Development of a *COMT* PCR multiplex to investigate resilience, anxiety and childhood trauma in a South African population

A thesis submitted in partial fulfilment of the requirements for the degree of Magister Scientiae in the Department of Biotechnology, University of the Western Cape

Sarah Jacobs

December 2020

Supervisor(s): Prof Sean Davison, Dr Fleur Howells (UCT), Dr Eugenia D'Amato

UNIVERSITY of the
WESTERN CAPE

University of the Western Cape

Private Bag X17, Bellville 7535, South Africa

Telephone: +27-21- 959 2255/959 2762 Fax: +27-21- 959 1268/2266

Email: 3135264@myuwc.ac.za

FACULTY OF NATURAL SCIENCE

GENERAL PLAGIARISM DECLARATION

Name: Sarah Jacobs

Student number: 3135264

1. I hereby declare that I know what plagiarism entails, namely to use another's work and to present it as my own without attributing the sources in the correct way. (Refer to University Calendar part 1 for definition)
2. I know that plagiarism is a punishable offence because it constitutes theft.
3. I understand the plagiarism policy of the Faculty of Natural Science of the University of the Western Cape.
4. I know what the consequences will be if I plagiarise in any of the assignments for my course.
5. I declare therefore that all work presented by me for every aspect of my course, will be my own, and where I have made use of another's work, I will attribute the source in the correct way.

Signature: 

Date: 04 December 2020

Acknowledgements

To Fleur Howells, Shareefa Dalvie and Kimberly Williams for being my mentors and guiding me through this degree, my thesis completion would have not been possible without any of you. A special thank you to Fleur Howells for welcoming me into your research group, for teaching me aspects of neuroscience. Thank you for your patience with me, and pushing me towards the completing of this degree.

To Sean Davidson for introducing me to a new field and opening up doors of opportunity.

To my parents and sisters for the continuous motivation and words of encouragement when it was needed most.

To my husband, Sulaiman, you've been there from the very beginning. Thank you for believing in me when I didn't, for ensuring that I did my best & for supporting me in every aspect while I focused on finishing my degree.

To the post graduates at UWC whom have all helped me in various aspects and stages of my degree; Brendon Pearce for teaching me SNaPshot, Mohamin Kasu for ensuring I had what I needed in the lab, Jihaan Adonis, Gerhard Basson and Kyle Phillips for helping me not just with my work but for being an incredible supportive group of friends.. Safia Zainudeen for the late nights and staying up with me to get through the writing process. Gadija Kaprey and Gadean Brecht for taking on my work while I focused on my thesis.

To the National Research Foundation for funding me for the full duration of my degree.

Table of Contents

Abstract

Anxiety, resilience and childhood trauma can be categorized as functional behavioural categories, with a wealth of research behind each. The research approach adopted for each, in most cases, is either from a genetic or neuropsychological standpoint, with few studies combining both to better understand all three functional behavioural categories as a multidimensional construct.

A number of candidate genes have been identified as markers for anxiety, resilience and childhood trauma, of which Catechol-methyl-transferase (*COMT*) and several respective single nucleotide polymorphisms (SNPs) are included. Although *COMT* SNPs have been linked to at least one of the functional categories, with a handful of haplotypes identified, to our knowledge no study has investigated the combination of SNPs selected for this study (rs6269, rs4818, rs4680, rs4633, rs737865, rs2075507) as a possible haplotype, specifically in a South African population. The use of SNaPshot for the genotyping of genes is an efficient and reliable means of identifying genotype frequencies and haplotypes in large sample groups, yet when selecting more than two SNPs of interest, the development of a multiplex assay is ideal. The first aim of the study was to design and optimize a multiplex assay to genotype several *COMT* SNPs. The primer design, multiplex optimization and SNaPshot conditions used showed good working parameters that can be utilized and further improved by optimization.

Self-report measures are widely used to measure psychiatric disorders, such as anxiety, and has also been used for the measurement of resilience and childhood trauma. With each functional behavioural category well investigated in its respective domain, there is a need to investigate all three as a collective in a South African population due to the high rate of anxiety and childhood trauma exposure in communities. The second aim of the study was to investigate the prevalence of anxiety, resilience and childhood as functional behavioural categories in the full South African sample group; and the role of sex, through established self-report measures and respective normative data. Additionally, this carried over into investigating the correlation between anxiety, resilience and childhood trauma as a multidimensional construct in both the full South African sample and between sexes. There is a clear relationship which exists between all three functional behavioural categories, as they show a correlation in various dimensions independent of one another. Higher anxiety levels amongst females were reported, with no difference between sexes for resiliency.

The empirical data collected from both *COMT* SNP and self-report measures for male and female where explored and reviewed against current literature for better understanding and insight into the association of *COMT* SNPs with anxiety, resilience and childhood trauma in a South African population. The results of this study to understand the complexity and association of all three functional behavioural categories as a multidimensional construct will be invaluable and may assist in the identification of possible risk factors which are essential for the promotion of better mental health in society.

Keywords: Catechol-methyl-transferase (*COMT*), single nucleotide polymorphisms (SNPs), anxiety, resilience, childhood trauma

List of Abbreviations

5-HTTLPR	Short allele of the serotonin transporter-linked polymorphic regions
ACE	Adverse Childhood Experiences
AD	Aldehyde dehydrogenase
ADHD	Attention-Deficit-Hyperactivity Disorder
ANS	Autonomic nervous system
AR	Aldehyde reductase
ARM	Adult Resilience Measure
ASW	African Ancestry in Southwest
BAI	Beck Anxiety Inventory
BAS	Behavioural activation system
BIS	Behavioural inhibition system
BNDF	Brain-derived neurotropic factor
bp	Base pairs
BP	Bipolar disorder
BRS	Brief Resilience Scale
CDC	Centres of Disease Control and Prevention
COMT	Catechol-o-methyl transferase
CSA	Childhood sexual abuse
CTQ	Childhood trauma questionnaire
CYRM	Child Youth Resilience Measure
ddNTPs	Dideoxynucleotide triphosphates
dH_20	Distilled water
DHMA	3,4-dihydroxymethamphetamine
DHPG	3,4-dihydroxy phenyl glycol
DOPAC	3,4-dihydroxyphenylacetic acid
DOPAL	3,2-dihydroxyl phenylacetaldehye
DOPEGAL	3,4-dihydroxy phenyl glycolaldehyde
DOPET	3,4-dihydroxy phenyl ethanol
DRD_2	Dopamine receptor D_2
EtOH	Ethanol
Exo	Exonuclease

GAD	Generalized anxiety disorder
HPLC	High pressure liquid chromatography
HRM	High Resolution Melt
HVA	Homovanillic acid
IDT	Integrated DNA Technology
LWK	Luhya in Webuye, Kenya
MAF	Minor allele frequency
MAO	Monoamine oxidase
MB-COMT	Membrane bound catechol-o-methyl transferase
Met	Methionine
MHPG	Vanylglycol
MOPEGAL	3-hydroxy-4-hydroxyphenyl glycolaldehyde
MtDNA	Mitchodonrial DNA
MYH15	Myosin heavy chain 15
NaCl	Sodium chloride
NCBI	National Centre for Biotechnology Information
NEB	New England Biolabs
NTRK2	Neurotrophic receptor tyrosine kinase 2
NPY	Neuropeptide Y
OCD	Obsessive-compulsive disorder
PCR	Polymerase chain reaction
PD	Panic disorder
PFC	Prefrontal cortex
PTSD	Post-traumatic stress disorder
rfu	Relative fluorescence units
SA	South Africa
SAD	Social anxiety disorder
SAP	Shrimp alkaline phosphate
SASH	South African Stress and Health study
SAQ	Social Anxiety Questionnaire
SBE	Single base extension
S-COMT	Soluble catechol-o-methyl transferase
SIT	Stress inoculation theory
SLC6A4	Serotonin transporter gene

SNPs	Single nucleotide polymorphisms
SPF	Scale of protective factors
STAI	Spielberger State-Trait Anxiety Inventory
STRs	Short tandem repeats
sz	Fragment size (bp)
T_a	Annealing temperature
T_m	Melting temperature
TMEM106B	Transmembrane protein 106B
UBPL	Upper-bound poverty line
Val	Valine
VMA	Vanillylmandelic acid
YRI	Ypruba in Ibadan

List of Figures

List of Tables

CHAPTER 1

LITERATURE REVIEW

1.1 Introduction

The following chapter serves to outline the genes, namely, catechol-methyl-transferase (*COMT*) and several respective single nucleotide polymorphisms (SNPs) which have been linked to anxiety disorders. In addition, the need for genetic and psychological tools to understand the way in which anxiety associates with an additional two functional behavioural categories; resilience and childhood trauma is also discussed.

The chapter has been sub-sectioned into two parts; the first focuses on *COMT* linked SNPs implicated in anxiety disorders, supported by the development and use of a mini-sequencing assay, SNaPshot for identification of these SNPs. The second focuses on the psychological aspect of anxiety, resilience and childhood trauma, and the use of self-report measures to understand anxiety, resilience and childhood trauma as functional behavioural categories.

Additionally, rationale for the complexity of association of all three functional behavioural categories and the need for the use of both genetic and psychological measures, such as self-report scales to understand it as a multidimensional construct is provided.

1.2 Genes implicated in psychiatric disorders

Decades of research has yielded a number of genes linked to an assortment of psychiatric disorders. Research has recognized that the majority of psychiatric disorders are passed down in families, which has led to the belief that there is genetic basis for some disorders. Many of these disorders have shared symptoms which suggest that at a biological level similarities may also be shared (NIH, 1998). Psychiatric genetics is defined as a subfield of behavioural neurogenetics and genetics, which studies the role of genetics in the development of mental disorders. Numerous new approaches to understanding psychiatric genetics have been developed over the years; of which enhanced expression, brain imaging and genomic tools have surfaced. Tagging SNP-based association studies and whole-genome association studies have become one of the most widely used tools for this kind of psychiatric genetic research in recent years (Züchner *et al*., 2007).

Genetic variations have been identified in a number of genes, with most associated to monoamine pathways (Züchner *et al*., 2007). Monoamine refers to specific neurotransmitters, namely dopamine, noradrenaline and serotonin. Dopamine and noradrenaline are often referred to as catecholamines due to their chemical structure, as they share a benzene ring and two hydroxyl groups (Goldstein, 2010). Catecholamines function within the central nervous system as neurotransmitters and peripherally as hormones in a range of physiological processes. The deficit of these catecholamines within the central nervous system have been linked to a variety of psychiatric and medical disorders, with the degradation of cathecholamine and its dysfunction being of particular interest in research in mental health disorders (Eisenhofer *et al*., 2004)

Degradation of catecholamines is a four-step process, depicted in **Figure 1.1.** The process starts with the deamination, which is catalysed by monoamine oxidase (*MAO*), forming deaminated aldehyde intermediate. Dopamine is converted to 3,2-dihydroxyl phenylacetaldehyde (DOPAL), while norepinephrine and epinephrine are both converted to 3,4-dihydroxy phenyl glycolaldehyde (DOPEGAL). This is followed by the formation of acid metabolites by two enzymes, namely aldehyde dehydrogenase (AD) and aldehyde reductase (AR). AD coverts DOPAL to 3,4-dihydroxyphenylacetic acid (DOPAC) and DOPEGAL to 3,4-dihydroxymethamphetamine (DHMA), while AR converts DOPAL to 3,4-dihydroxy phenyl ethanol (DOPET) and DOPEGAL to 3,4-dihydroxy phenyl glycol (DHPG). Due to dopamine and DOPAL lacking a beta-hydroxyl group, DOPAL binds to AD, which leads to the formation of DOPAC. Due to epinephrine, norepinephrine, and DOPEGAL all consisting of a beta-hydroxyl group, DOPEGAL binds to AR, leading to the formation of DHPG. The next step in degradation is methylation, which is catalysed by *COMT*. DOPAC is converted to homovanillic acid (HVA), the end-product of dopamine degradation, while the DHPG is converted to vanylglycol (MHPG). *COMT* may also convert norepinephrine to normetanephrine, which is converted to 3-hydroxy-4-hydroxyphenyl glycolaldehyde (MOPEGAL) by *MAO* (indicated in red in **Figure 1.1**). The final step is the formation of vanillylmandelic acid (VMA) which is catalysed by acetaldehyde dehydrogenase. MHPG is converted to MOPEGAL and MOPEGAL

converted to VMA, the end-product of epinephrine and norepinephrine degradation (Kamal, 2019).

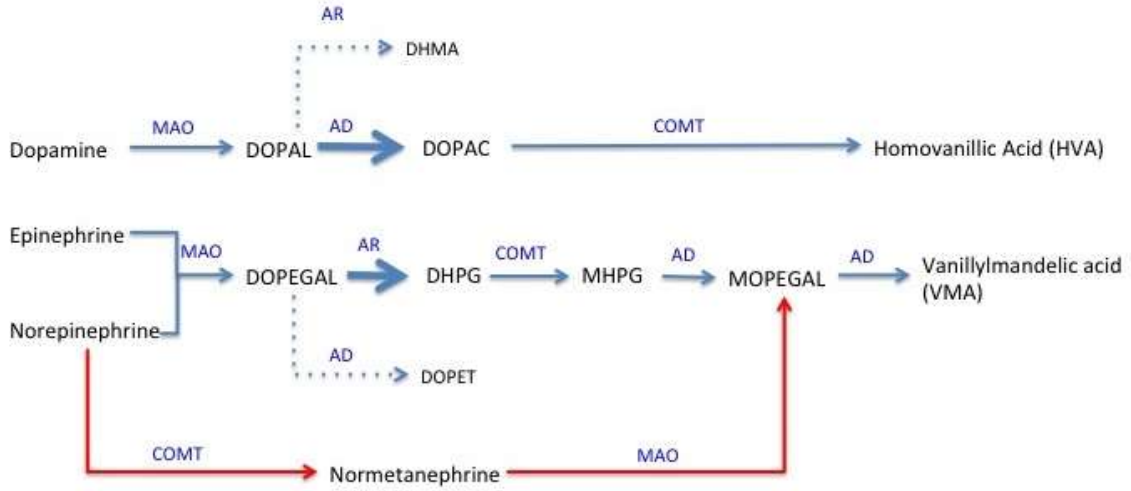

Figure 1. 1 The degradation pathway of catecholamines. The degradation of catecholamines, namely dopamine, epinephrine and norepinephrine is a four-step process which involves a number of enzymes (Kamal, 2019).

The role of each of these enzymes and their respective genes, specifically *MAO* and *COMT*, have been studied for their association in the degradation of catecholamines linked to psychiatric disorders, due to their influence in the metabolizing and inactivation of dopamine (Rosengren, 1960; Volavka *et al*., 2004). *COMT* has become a therapeutic target due to its regulatory link to numerous pathologies, including cardiovascular disease and neurophysiology associated disorders (Hall *et al*., 2019).

1.3 Catechol-O-methyltransferase SNPs as a genetic predisposition for psychiatric disorders

The catechol-O-methyltransferase (*COMT*) gene encodes for the COMT enzyme which regulates synaptic catecholamine neurotransmitters such as dopamine and epinephrine (Qayyum *et al*., 2015). The 27.22kb *COMT* gene, located on chromosome 22q11.2, has two isoforms which are expressed from two promoters; namely soluble (S-COMT) and membrane-bound (MB-COMT). S-COMT is expressed in most tissue types, including the blood and kidneys, whereas the MB-COMT isoform is mainly expressed in the brain. MB-COMT has been shown to play a role in the regulation of extracellular dopamine levels within the prefrontal cortex of the human brain and accounts for 70% of total *COMT* polypeptides in the brain (Tenhunen *et al*., 1994). MB-COMT has been found to be more effective in the

metabolism of catecholamines, in comparison to S-COMT, due to its 10-fold higher affinity for dopamine and norepinephrine, which has made it relevant to research in both psychiatric and cognitive phenotypes (Rothe, 1992).

Due to *COMT* metabolizing catecholamines, it has been linked to a range of brain function and dysfunction such as working memory and emotional processing, and has therefore been suggested to be associated with a number of psychiatric disorders including anxiety (Hettema *et al*., 2008; Stein *et al.*, 2005; Baumann *et al.*, 2013). A recent study which investigated the relationship between working memory performance, anxiety and stress, found increased anxiety (state and trait) to be negatively associated to poor working memory performance (Lukasik *et al*., 2019). A similar study which looked into emotional dysfunction in the context of anxiety found that emotional dysfunction predicted an increase in anxiety symptoms (McLaughlin *et al*., 2011).

The functional *COMT* polymorphism, Val ^{158} Met (rs4680), has been found to account for a four-fold variation in COMT enzyme activity where the Val allele increases *COMT* activity resulting in a decrease in cortical dopamine levels (Gruss *et al*., 2016). Then low activity of Val ^{158} Met has been associated with improved working memory (Enoch *et al*., 2009) as well as an increased risk for a range of anxiety disorders (Chen *et al*., 2004; Smolka, 2005). The use of genetic imaging studies allows for researchers to better understand, with the use of neuroimaging techniques, the way in which genetic variations affect the brain function or structure for the purpose of understanding how it impacts behaviour or disease phenotypes (Steckler and Salvadore, 2013). The findings of Chen *et al*.(2004) and Smolka (2005) align with the results reported from imaging genetic studies which show a change in the processing of emotional cues in anxiety related cortical and subcortical brain regions in the presence of *COMT* (Chen *et al*., 2004; Domschke *et al*., 2012; Smolka, 2005). Heritable variation within the neurotransmission of dopamine associated with *COMT* polymorphisms leads in an increased reactivity and connectivity within the cortical limbic circuits of the human brain. This observation has been suggested to be a reflection of a genetic predisposition for the processing of affective stimuli, a possible mechanism for anxiety arousal (Drabant *et al*., 2006).

A 2005 longitudinal study which investigated the association of the *COMT* Val ^{158} Met polymorphism and anxiety, reported that individuals who were carriers of the Met ^{158} Met genotype were twice as likely to also report anxiety related behaviour, namely phobic

avoidance and panic attacks, suggesting an association between the Val 158 Met polymorphism and specific expressions of anxiety behaviour. They further reported females to be of higher risk as stratification by sex showed the risk effect for the Met allele amongst females only (Olsson *et al*., 2005).

There are a number of studies which have reported an association between *COMT* and anxiety disorders, with mixed findings. Some studies have reported the Val allele to be associated with panic disorder (PD), specifically in Caucasian females (Domschke *et al*., 2004; Rothe *et al.*, 2006). McGrath and colleagues have also reported the Val allele to be associated with an increased risk for phobic anxiety, which aligns with the study by Olsson and authors (McGrath *et al*., 2004). On the other hand, studies which consisted of mostly Asian population groups found no association with the Val allele and anxiety disorders, suggesting that population genetics is an important factor when investigating the association of genes such as *COMT* to psychiatric disorders (Ohara *et al*., 1998, Samochowiec *et al.*, 2004)

1.4 The use of SNPs for genetic variation

SNPs are single base changes at specific positions within the human genome and are the most common source of genetic variation, as they occur in both coding and non-coding regions of the human genome and are therefore high in abundance (Butler, 2012; Rafalski, 2002). Variation within DNA sequences can directly and indirectly impact the way in which individuals develop and react within an environment (Angers *et al*., 2010). A large number of SNPs have been identified and mapped onto the human genome; and have been used as markers for the study of genetic disorders (Wright *et al*., 2003). Due to the low mutation rate, SNPs are ideal genetic markers for a number of reasons. With the use of common applications such as polymerase chain reaction (PCR), product can easily be produced (Edwards *et al*., 2007). The required size for SNPs can be less than 100bp in size, therefore researchers are able to recover information from degraded DNA samples easier using SNPs instead of short tandem repeats (STRs). In principle SNPs may also be multiplexed on a higher level, as detection methods such as array hybridization are not limited by dye labels and size range. Due to size based separation not being required for SNPs the data processing and analysis on SNPs is more fully automated. There is also no stutter artefact in allele association of SNPs, therefore allele call interpretation is more simplified than in STR and allows for easier identification of peaks (Butler *et al.*, 2007).

Most SNPs have two possible alleles, referred to as being bi-allelic, which means they have three possible genotypes. If the alleles for a given SNP locus is X or Y for example, where X can represent an A, C, G or T nucleotide and Y could also be an A, C, G or T nucleotide, then the three possible genotype outcomes would be XX, YY or XY. These genotypes are referred to as being homozygous (XX or YY) or heterozygous (XY), which can present a challenge in mixture interpretation as it may not always be easy to differentiate between a true heterozygote or a combination consisting of two homozygotes or a heterozygote and homozygote combination. (Butler *et al*., 2007).

In DNA typing applications using SNPs one of the most difficult challenges is the inability to amplify sufficient SNPs in multiplexes from small amounts of DNA simultaneously due to the fact that single bi-allelic SNPs produce less information than STRs which are multi-allelic. However advancements in the use of multiplexing in PCR amplification and assays have allowed researchers to amplify and analyse multiple SNPs simultaneously (Sanchez *et al*., 2006).

1.5 The SNaPshot assay for SNP detection

There have been a number of advancements and developments in technology used for SNP analysis, each with its own strengths and weaknesses, which include sequence analysis and microchip hybridization. A crucial factor in SNP assays is the capability of examining numerous markers at once. Both pyrosequencing and TaqMan assays have limited multiplexing capabilities, yet minisequencing assays such as SNaPshot or Luminex allow for the multiplexing of more than several SNP markers in one sequence run. SNaPshot has since been widely used in forensic labs for the detection of mitochondrial DNA (mtDNA), Y-chromosomes and autosomal marks on ABI 310 or 3100 capillary sequencers common to forensic labs (Butler, 2012).

SNaPshot is a minisequencing method which uses allele-specific primer extension and fluorescent dye labelled dideoxynucleotide triphosphates (ddNTPs) to produce electropherograms as a visual representation of results per sequencing run. There are three main steps in SNaPshot, these are, amplification, primer extension and analysis, as shown in **Figure 1. 2**. The region around each SNP of interest is first amplified using PCR, with the amplicons either pooled after singleplex PCR or amplified simultaneously in multiplex PCR. A clean-up

is then performed to remove all remaining dNTPs and primers from the PCR using Exonuclease (Exo) and shrimp alkaline phosphate (SAP). Exo removes all single stranded primers, while SAP removes all unincorporated dNTP building blocks. This clean up step is crucial in preparation for minisequencing as it ensures the primers and dNTPs do not interfere with primer extension. The primer extension is performed by the addition of extension primers to the SNP of interest with a combination of four possible dideoxynucleotides triphosphates (ddNTPs) and a polymerase. Each of these ddNTPs has a unique fluorescent dye label. The design of the SNP extension primers allows for it to anneal directly to the SNP site, which allows the addition of a single ddNTP integrated into the nucleotide found at the SNP site. A second clean-up with SAP is performed on the PCR products to remove unincorporated fluorescent ddNTPs. An incomplete or failed clean up may lead to dye artefact in the electropherogram which causes the SNP allele peaks being measured to be unclear (Butler, 2012).

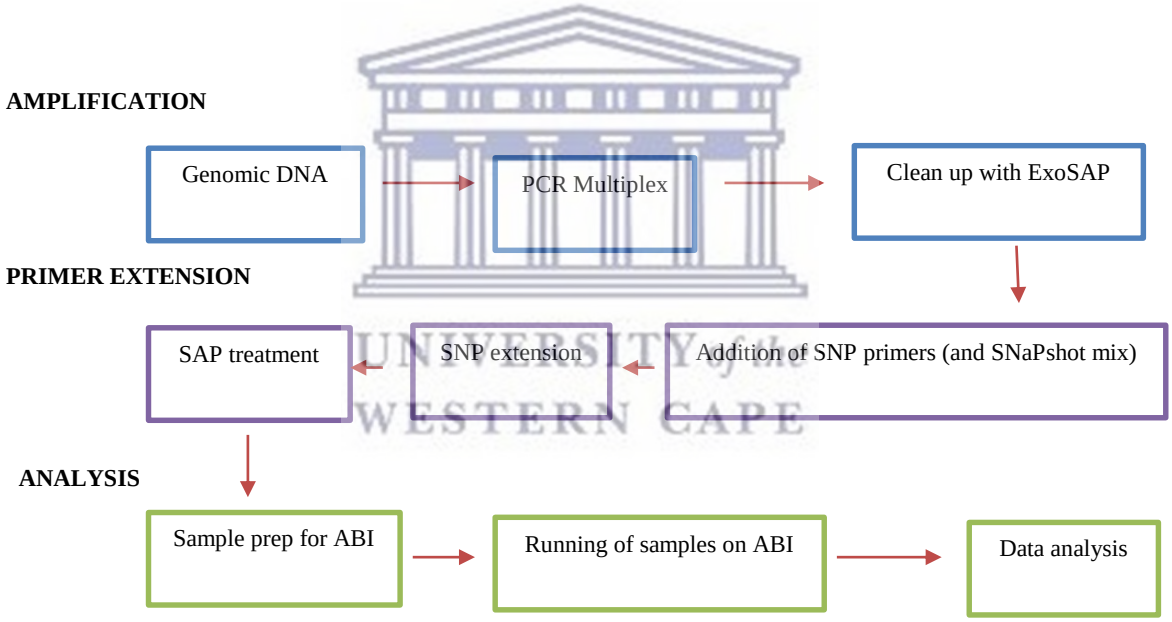

Figure 1. 2 The process of minisequening assays. The three main steps in allele-specific primer extension SNP detection, namely amplification, primer extension and analysis by means of minisequecning or SNaPshot (Diagram adapted from Butler, 2012).

Electrophoretic machinery such as the ABI have five dye detection capabilities which allow for the addition of an internal size standard to be added to the fifth channel. This ensures run to run migration differences are automatically corrected. Each nucleotide has a dye colour specific to it, A is green, G is blue, C is yellow or black and T is red. This means that a green peak on an electropherogram would be indicative of an A (ddATP) incorporated by the

polymerase at the site of the SNP of interest. A homozygous allele would therefore appear as single peaks while heterozygous alleles will appear as two overlapping peaks (Butler, 2012). SNaPshot is a fairly robust assay which allows for the analysis of multiple primers by the addition of nucleotides to the 5'- end, ensuring each primer differs. Poly (T) tails are used for this, with 3-5 base differences between each subsequent primer to ensure each locus is determined properly and differentiated from the others (Vallone *et al*., 2004).

1.6 The spectrum of anxiety

Anxiety is the term used to categorize a range of disorders which cause fear, nervousness and worry; ranging from mild to severe cases. In 2009, Herman and colleagues reported that anxiety disorders fall under the most prevalent of lifetime disorders in South Africa, contributing to 15.8% of overall mental disorders (Herman *et al*., 2009). There are several classified anxiety disorders, which include panic disorder (PD), obsessive-compulsive disorder (OCD), post-traumatic stress disorder (PTSD), phobias, generalized anxiety disorder (GAD) and social anxiety disorder (SAD) (DSM-5; American Psychiatric Association, 2013).

The above mentioned presentations of anxiety can in part be grouped into two distinct categories; namely state or trait anxiety, as first suggested by Spielberger in 1966 (Spielberger, 1989). State anxiety, classified as a psychophysiological state, is characterized by cognitive worry and autonomic-emotional driven by the autonomic nervous system (ANS). The ANS and hypothalamus aids in regulation of generation, expression or response to emotional signals (Levenson, 2014). Trait anxiety, classified as a personality trait, is characterized by social evaluation, physical danger, ambiguous and daily routines (**Figure 1.3**). Simple daily tasks such as crossing a busy intersection during peak hour traffic or walking pass an aggressive barking dog can cause trait anxiety, which will vary between individuals depending on how strong their sense of anxiety is. Both state and trait anxiety vary between individuals, as each of the above mentioned characteristics are individual dependent (Leal *et al*., 2017; Endler and Kocovski, 2001).

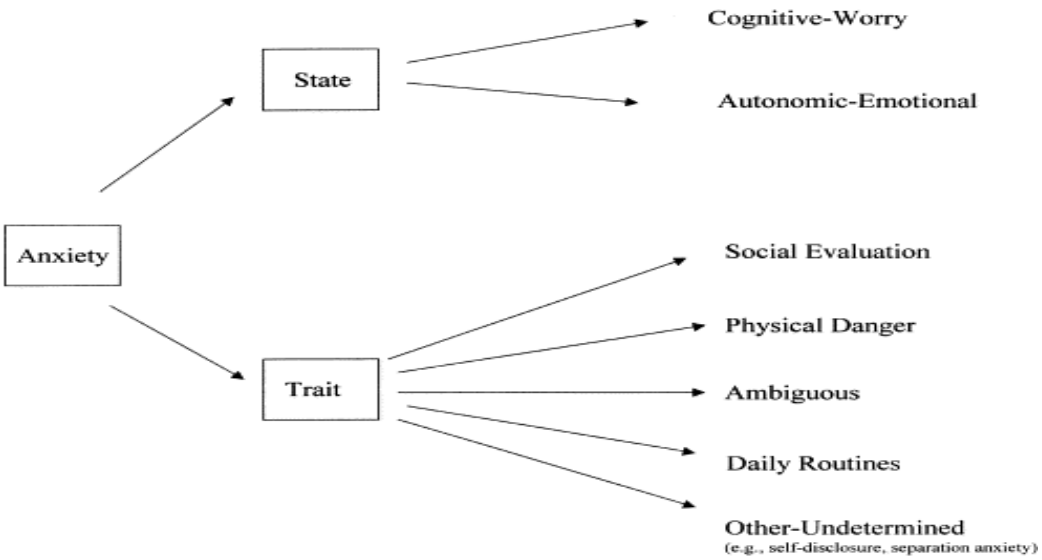

Figure 1. 3 Anxiety is defined by State or Trait. Anxiety is categorized into two multidimensional constructs; state or trait anxiety, with each characterized by respective common characteristics (Endler and Kocovski, 2001).

State anxiety occurs when an individual is in a situation that can be of threating demand and/or danger, which causes an unpleasant emotion to arise. For the individual to experience this sort of emotion to the extent it causes state anxiety, a cognitive appraisal of the threat must take place prior (Shcwarzer, 1997). Trait anxiety is the individual difference, in the way the individual responds with state anxiety to a given threatening situation (Lazarus, 1991).

There exist a number of well supported and reputable self-report measures which are used to measure anxiety in young adolescents. Of these scales the Spielberger Trait-State Anxiety Inventory (STAI), Beck Anxiety Inventory (BAI) and Social Anxiety Questionnaire (SAQ) are included.

1.7 Childhood trauma exposure in South Africa

It is well known that childhood trauma, especially in context of abuse, is rife in South African populations (Ward *et al,* 2012). Pawelczyk (2012) reported that between March 2011 and April 2012 the reported crimes against children in South Africa were 54,000, which is considered to be a fraction of the real number due to the high number of crimes against children which go unreported (Pawelczyk, 2012). An earlier study which looked at high levels of exposure to not only trauma but violence in both adolescents and children in South Africa found between 40% and 100% reported trauma and violence, respectively (Suliman *et al*., 2005). The impact of

childhood trauma can have detrimental effects on the mental health of young adolescents, with current literature suggesting that childhood abuse leads to an increased risk of anxiety in later life (Norman *et al*., 2012). Exposure to childhood adversity in the forms of traumas such as physical abuse impacts the transition into adulthood for an individual due to its impact on social factors; as well as physiological and neurobiological changes which are a result of chronic stressors (Patton *et al*., 2014). As suggested by meta-analyses physical sexual and emotional abuse have higher rates amongst sub-Saharan African children than in other settings (Stoltenborgh *et al*., 2015).

Being exposed to other variances of traumatic events which include abuse, neglect, the loss of a loved one, relationship problems and psychosocial stress factors have been to linked to mental health disorders in South Africans (Gregorowski and Seedat, 2013). In a study carried out by the Child Mental Health Unit at the Free State Psychiatric Complex to determine the profile of stressors which lead to mental disorders in 669 children and adolescents, 64.1% had reported experiencing social stressors and 19% reported experiencing psychological stressors, with 18.5% diagnosed with anxiety disorders (Calitz *et al*., 2012). Social stressors include domestic abuse, substance abuse within the family, financial struggles, and lack of support, parental separation, and problems within relationships and at school (Wheaton, 1999). Psychological stressors include emotional, physical and sexual abuse, isolation, low self-esteem, rejection, feelings of hopelessness and worthlessness (Monroe and Slavich, 2007).

In 2009, Leoshut investigated the influence of violence and crime on resilience in South African sample of 4 391 youth aged 12 to 22 years, reporting that 41% had experienced a minimum of one crime, 14.4% experienced some form of assault and 3.6% experienced sexual assault, concluding that individuals between 12 and 22 years are more likely to either be the victim or perpetrator (Leoshut, 2009). In the face of childhood adversity, a total of nine key resilience factors were identified by Leoshut (2009), which are believed to enhance resilience in younger individuals. These factors are education, gender, non-violent family environment, non-exposure to criminal role models, substance abstinence, interaction with non-delinquent peers, victimisation, neighbourhood factors and attitudes intolerant of violence and antisocial behaviour (Leoshut, 2009). Supporting the results reported by Leoshut (2009), in another study consisting of a sample group of 669 South African children and adolescents, 64.1% experienced social stressors, while 19% experienced psychological stressors and 18.5% of the sample were diagnosed with anxiety disorders (Calitz *et al.,* 2012). Research also suggests a

correlation between childhood trauma and psychiatric disorders in later life such as anxiety (van der Kolk, 2005).

It has been clearly established through decades of research that exposure to childhood trauma increases the risk of developing psychological or functional disorders during adulthood. However, adults with a history of childhood trauma that remain psychologically healthy and display no signs of a psychological disorder, deeming these individuals as being resilient have also been reported. Despite the research, the psychological processes of resilience is still a controversial topic. An important consideration when investigating childhood trauma, resilience and psychological disorders, is weather resilience results from childhood trauma or if resilience is in fact a common characteristic many individuals possess with or without exposure to traumatic life events (Phillpe *et al*., 2011).

Investigating childhood trauma exposure in South Africa has been of interest among researchers in mental illness disorders due to the strong association between adverse childhood experiences and psychological disorders such as anxiety and post-traumatic stress disorder (PTSD). There are a number of self-report measures which have been used to assess and measure childhood trauma exposure of with the Bernstein Childhood Trauma questionnaire and Adverse Childhood Experiences most commonly used (Baglivio *et al.*, 2014, Hernandez *et al.*, 2013; Karos *et al.*, 2014).

1.8 Resilience within South African communities

Rutter (1987) defined resilience as singular variations in the way in which individuals respond to stress and adversity. In South Africa these adversities include various forms of abuse, violence, poverty and deprivation, which are more frequent in low-income communities and lead to an increased chance of developing negative behavioural, health and cognitive conditions (Rutter, 1987; Duncan *et al*., 1994). Despite the many adverse conditions an individual may face, these may be overcome and lead to the development of resiliency, allowing for one to thrive in destructive environments (Walsh, 2006). A 2012 publication by Theron focused on resilience studies conducted specifically in South Africa between the years 1990 to 2011. They reported that majority of studies have been inconsistent, with studies prior to 2009 found to be insufficient due to non-representative and small sample groups, as well the use of non-specific resilience measures (Theron, 2012).

Based on the work by Reivich and Shatte (2002), there are seven areas of resilience identified; these are emotional awareness and regulation, impulse control, realistic optimism and thinking style, flexible thinking to problem solve, self-efficiency and self-accountability, empathy and reaching out. The common misconception surrounding resilience and emotion is that resilient individuals are "tough" and do not express emotion during adversity when in fact resilient individuals have a good understanding of their emotions. When faced with a difficult situation, resilience individuals are able to identify and manage the range of emotions experienced in the reality of an adverse situation, making them emotionally aware (Reivich and Shatte, 2002).

Impulse control is defined by the ability an individual has during adversities to control his/her actions, behaviour and emotions. Those that have higher resilience levels are able to manage ambiguity, reducing the risk of impulsive decisions. Realistic optimism is another key component of resilience. Research shows optimistic individuals are reported to be more productive, have better relationships, are better problem solvers and succeed more in key areas of their life. Individuals that are more focused on positive aspects of adversity are able to manage the negative aspects easier (Reivich and Shatte, 2002).

Resiliency requires an individual to think flexibly in order to view a problem from different perspectives, which lead to multiple solutions to a problem; therefore those that are deemed resilient are able to problem solve easily with a flexible train of thought. Another key component to resilience is accountability. Those that have a high daily resilience level personally take responsibility for their actions and decisions; and are confident in their decisions and are able to connect with others. These individuals are able to view a situation from another perspective, even if it disagrees with his/her beliefs, therefore making the individual more empathic. The last area of resilience is reaching out, which is defined as the extent to which an individual is able to confide in others and ask for support when needed (Reivich and Shatte, 2002).

There is a wealth of information which supports the notion of an interaction between risk and protective processes, functioning at different stages of development throughout the course of an individual's life span with these protective processes contributing to resilient outcomes of which general wellbeing is included (King and Madesen, 2007; Windle, 2011). An important factor to take into consideration in resilience studies is the sociocultural and economic context,

as various researchers conclude that these contribute to adverse experiences (Carrey and Ungar, 2007). Numerous studies have suggested resilience to operate and exist at a community level, which includes both psychological and sociocultural aspects (Ahmed *et al.*, 2004; Sonn and Fisher, 1998; Lyons *et al.*, 1998). The majority of studies on resilience have been based in Europe and USA, where the overall household income and resources are much higher than South Africa (Betancourt *et al*., 2011).

There exist a number of well-established self-report resilience scales, used to measure resilience within individuals with most designed and developed to include the general resilience dimensions. These dimensions include self-awareness care, positive relationships and purpose; with a clear relationship between all three dimensions (Jones, 2019). Self-awareness involves the emotion, cognition and behaviour of an individual which focuses attention on the understanding of one's thoughts and feelings, influencing self-purpose and the way in which relationships evolve (Morin, 2011). It can therefore be considered to be important in the process of stimulating adaptive and self-directed change, with self-awareness thus translating to resiliency (Caerver and Scheier, 1998). There are many associations found between personality, intelligence and resilience, these include a connection between higher personal competence and elevated emotional stability, with most studies making use of established resilience scales. These scales are suggested to be useful for assessing protective factors which can either inhibit or act as a buffer against psychological orders such an anxiety (Friborg *et al.*, 2005; Windle *et al.*, 2011).

1.9 Relationship between anxiety, resilience and childhood trauma

Protective factors may influence resiliency, which can be categorized as either intrinsic or extrinsic dimensions (Wolkow and Ferguson, 2001). Intrinsic factors refer to the potential an individual has to develop or accept the characteristics required to manage emotion or physiological situations which are promoted through the use of self-constructs such as self-control, self-esteem, self-efficiency, social competence, problem-solving skills and cognition processes. These characteristics can change with age and time (Werner and Smith, 1992). For physiological processes, the individual will make use of coping mechanisms when in a stressful situation which influences resilience (Wang *et al*., 1994). Extrinsic factors refer to circumstances whereby an individual's behaviour and thinking changes and adapts as a result of a new environment, which often occurs in families and the wider community (Wang *et al*.,

1994). Considering both the intrinsic and extrinsic dimensions of protective factors, the interaction of both leads to an increase in protective factors, which in turn boosts resiliency in an individual (Bichard, 2000). For example, a positive experience and performance at university can protect the self-esteem of an individual despite an adverse living condition at home.

Exposure to childhood trauma has been associated with an increased risk of developing anxiety related disorders, with a number of studies indicating that early childhood trauma exposure is associated with an increased risk of developing a range of anxiety disorders in adulthood, which include SAD and PSTD (Agid *et al*., 2000; Etkin and Wager, 2007; Fossion *et al*., 2014). Multiple early childhood trauma has also been linked to an increase in PTSD symptoms, with childhood trauma believed to be associated with lower resilience, especially in context of emotional neglect (Collin-Vézina *et al*., 2011; Simon *et al*., 2009). Abusive or neglectful experiences have been found to damage brain structure and functioning related to emotional processing by altering the main fibre tracts which link the prefrontal and subcortical regions of the brain, with childhood trauma inducing hyper-reactivity of the amygdala in response to negative emotional stimuli (Choi *et al*., 2009; Tottenham *et al*., 2011; van Harmelen *et al*., 2013). Adverse childhood experiences, such as emotional neglect increase both physiological and behavioural problems, which make the individual more vulnerable to mood problems and decreased resiliency (Al Odhayani *et al*., 2013).

However, not all those that experience childhood trauma develop anxiety or other mental health disorders, therefore are thought to be equipped with traits of resilience which serve as means of protection from developing these psychopathologies. Understanding how exposure to childhood trauma and abuse affects psychosocial development is important for understanding resilience (Collishaw *et al*., 2007).

Rutter (2007) proposed that adverse childhood exposure increases resilience in adolescents, even in the presence of a mental disorder, such as anxiety, a phenomenon explained by the stress inoculation theory (SIT) (Rutter, 2007). This theory refers to the prolonged exposure of unpredictable and uncontrolled stress which leads to long-term neurological impairment in individuals. This however is thought to increase in the efficacy and capabilities of managing and regulating future stressful events (Meichenbaum, 1989).

It has also been thought that childhood trauma exposure in some individuals could contribute to being less impacted by stress factors in later life (Campbell-Sills *et al.,* 2006). In the same study, Campbell-Sills *et al*. reported that higher levels of emotional neglect during childhood showed greater severity in symptoms if the individuals were less resilient. It was further found that those that had higher levels of emotional neglect during childhood coupled with higher resilience levels also scored the lowest in psychiatric symptoms (Campbell-Sills *et al.,* 2006). However in early life adversities the outcomes amongst individuals may differ significantly, which have shown to be affected by both duration and severity levels of adversity (Hovens *et al.,* 2012; Horn *et al*., 2016). Furthermore, in childhood and adolescent life, the developmental timing of adversities may have a differential impact on the lifelong health and well-being of an individual (Gee and Casey, 2015). This is a considerable factor as within South African communities, exposure to adversities such as poverty, abuse, neglect and violence ranges from childhood to adolescent life.

1.10 The need for genetic and psychological self-report measures to understand anxiety, resilience and childhood trauma as a multidimensional construct

Anxiety, resilience and childhood trauma have each been investigated as standalone functional behavioural categories' in psychological research settings (Dymond and Roche, 2009; Chorpita and Taylor, 2002; McLaughlin *et al*., 2020), as well as genetic respectively (Gottschalk *et al*., 2017; Morris-Rosendahl *et al*., 2002; Maul *et al.,* 2020 , Jiang *et al.,* 2019) yet to our knowledge no study has combined all three categories' in psychological and genetic fields to investigate the association and relationship between as a multidimensional construct.

Both resilience and anxiety have multiple interacting factors; which include genetics, epigenetics; as well as developmental environment and psychosocial factors, all of which may be impacted by exposure to childhood adversity (Southwick and Charney, 2012). Epigenetics refers to the functional modifications within the human genome that does not change the DNA sequence, but rather regulates gene expression and phenotypes. These differences can be a result of exposure to stressors during important periods of development, thereby influencing psychiatric disorders (Dudley *et al*., 2011). Both genetic and epigenetic factors interact directly and indirectly with one another to determine biological characteristics and regulation of neurochemicals and receptors. Environmental factors contribute to these by influencing the

biological characteristics of neurochemicals and receptors; and regulation processes through gene to environment interactions (Tsuang *et al.,* 2004). This contributes and may lead to possible adaptive changes in gene regulation, modulation of neurocircuits, as well as the shaping of psychological factors; which include cognitive processes, personality traits and active coping mechanisms; and behavioural changes that underline resilience and anxiety (Wu *et al*., 2013).

In 2006, Luthar *et al*. had brought to light the lack of studies which examine gene to environment interaction, suggesting for future research methods to consider focusing on outcome variables, especially in resilience (Luthar *et al.,* 2006). A 2016 study suggested that the active coping mechanisms, along with social support and post-traumatic stress disorder (PTSD) symptom severity in individuals, reported at the age of which a traumatic event has occurred can lead to the prediction of a positive-negative change in resiliency. The authors concluded by suggesting that more in depth and comprehensive assessments of resilience, the severity of childhood trauma exposure and other environmental stress factors will provide a better overall understanding of factors which contribute and either facilitate or deter the adaptions of social anxiety disorder (SAD) or PTSD in individuals (Brooks *et al*., 2016).

Multidisciplinary research to understand the relationship between anxiety, resilience and childhood trauma is an important consideration since resilience, with childhood trauma, could facilitate the development of modified target treatment and recovery for patients which have anxiety disorders (Marx *et al.,* 2017). Resilience can be considered to be an important tool in the treatment of those that suffer from anxiety disorders, especially amongst those that have been exposed to adverse childhood conditions and trauma (Connor and Davisdson, 2003).

To our knowledge there has been little investigation into the resilience of young adolescents with anxiety disorders, in the context of childhood trauma in South Africa, which serves as motivation for this condition to be investigated from both a genetic and psychological standpoint. It is for this reason, combining both genetic, namely genotyping of genes associated to the disorders of interest and psychological work, such as the use of established self-report measures; is required to better understand the relationship between resilience, anxiety and childhood trauma.

1.11 Study outline and objectives

Anxiety, resilience and childhood trauma have been well researched and investigated on their own in a number of population groups as functional behavioural categories, with some research investigating two of the respective categories simultaneously. With many similarities and associations between each, there is a need to investigate the relationship between all three, specifically in a South African sample group. This study consists of two main components The first is based on the use and application of genetic work for the development of a *COMT* multiplex assay to identify a *COMT* haplotype in a South African population. The second is based on the use of self-report measures to investigate three functional behavioural categories; anxiety, resilience and childhood trauma in a South African population, and the role of sex.

The aims of the study are therefore as follows;

- To design and optimize a multiplex assay for the genotyping of several *COMT* SNPs for the purpose of identifying a possible haplotype linked to anxiety, resilience and childhood trauma.
- To investigate the prevalence of anxiety, resilience and childhood as functional behavioural categories in the full South African sample group; and the role of sex through established self-report measures and respective normative data
- To investigate the correlations between anxiety, resilience and childhood trauma as a multidimensional construct in both the full South African sample and between sexes

CHAPTER 2

THE DESIGN AND OPTIMIZATION OF A WORKFLOW FOR THE IDENTIFICATION OF MULTIPLE *COMT* SNPS

2.1 Introduction

To date, 29 *COMT* SNPs have been identified, all located on the forward strand of chromosome 22. Due to their impact on *COMT* activity SNPs rs6269, rs4818, rs4633 and rs4680 have been of particular interest in research as they are used to define a low and high activity haplotype (Nacklet *et al*., 2009; Rotten *et al*., 2011). A haplotype is defined as a set of inherited polymorphisms found on the same chromosome (Glusman *et al*., 2014). The rs6269-rs4633-rs4818-rs4680 haplotype affects both the COMT protein levels and enzyme activity by means of modifying the mRNA secondary structure. This in turn leads to a number of phenotypes variations, as a result haplotype G-C-G-G leads to high levels, A-T-C-A will result in normal levels and A-C-C-G will result in low COMT protein levels and enzyme activity (Nackley *et al.*, 2006).

The SNPs of interest for this study, with their respective base changes, minor allele frequencies (MAF) and association to the respective functional behavioural categories; namely anxiety, resilience and childhood trauma are shown in **Table 2.1** These SNPs are namely, rs6269, rs4818, rs4680, rs4633, rs737865 and rs2075507. Previous studies which showed association between *COMT* variants and at least one of the functional behavioural categories directly or indirectly as either standalone variants or haplotypes were the basis for the SNP selection.

Table 2. 1 A list of the selected SNPs, respective base changes, minor allele frequencies (MAF) in African populations and linked functional behaviour categories

SNP	Base Change [a]	Ancestral [a]	Effect [a]	MAF[a]	Associations with functional behavioural categories
rs6269	A/G	G	5 prime UTR variant	0.371	PTSD (Zhang *et al*., 2018)
rs4818	C/G/T	G	Non-synonymous	0.170	No direct association (linked to emotional regulation)
rs4680	G/A	G	Missense variant	0.281	Anxiety, resilience, PTSD, childhood trauma (Hettema *et al*., 2008; McGrath *et al*., 2004; Kim *et al*., 2006; Kolassa *et al*., 2010; Boscarina *et al*., 2011; van Rooij *et al*., 2016)
rs4633	C/T	C	Non-synonymous	0.293	PTSD(Kwon *et al.*, 2020))
rs737865	A/G	A	Intron variant	0.126	Part of a 3 marker haplotype linked to anxiety (Wray *et al*., 2008; Stein *et al*., 2005)
rs2075507	G/A, G/T	A	Intron variant	0.640	No direct association (linked to working memory and executive functioning)

[a] Information extracted from Ensembl for AFR populations (https://www.ensembl.org/Homo_sapiens/Info/Index)

SNP, rs6269 has been associated with childhood maltreatment and a number of psychiatric disorders including PTSD (Zhang *et al*., 2018). In the Zhang *et al*., study the G variant of rs6269 was linked to a COMT haplotype (rs6269-rs4633-rs4818-rs4680: G-C-G-G) for increased risk of PTSD. Nackley *et al*., (2006) reported that a two functional SNP haplotype (rs6269 and rs4818) produce diplotypes ValA/ValA, ValA/Met, ValA/ValB or Met/Met, ValB/Met, and ValB/ValB, which have been ranked from the highest to lowest *COMT* enzyme activity (Nackley *et al*., 2006). In 2009, Barnett and colleagues showed a curvilinear association of the rs6269/-rs4818 haplotype with verbal inhibition and working memory (Barnett *et al*., 2009)

SNP, rs4818 has a synonymous base change and therefore does not affect the amino acid, with a C/G substitution (Leu/Leu) at codon position 86 of S-COMT or codon 136 of MB-COMT (Roussos *et al*., 2008). This SNP has been shown to play a role in cognitive functioning (SNPedia, 2019), with the G variant associated to increased *COMT* activity and therefore reduced PFC dopamine activity (Roussos *et al*., 2008). The effect of the C/G substitution in

PFC functioning impacts emotionally informed decision among individuals. High PFC levels of dopamine lead to negative choices when decision making is dependent on the processing of emotional feedback, while low PFC levels of dopamine impact positively in emotionally informed decision making (Roussos *et al*., 2008). Individuals with anxiety disorders are suggested to be characterized by maladaptive patterns of emotional regular, with a significant association between difficulties in emotional regulation and anxiety symptoms (Cisler *et al*., 2010). Although no studies have reported an association between rs4818 and anxiety as a standalone functional variant, its impact on dopamine activity indirectly impacts cognitive function and emotional regulation, which are associated to anxiety disorders.

The SNP, rs4680, commonly referred to at Val158Met due to its substitution of amino acid Val158 for Methionine is the most well studied *COMT* polymorphism due to its reported effect on executive functions, working memory and cognitive flexibility (Goldberg *et al.*, 2003). This SNP, which involves a base change in amino acid from valine (GTG/Val) to methionine (ATG/Met), at either codon 108 (S-COMT) or 158 (MB-COMT), directly influences the regulation of COMT enzyme activity (Lachman *et al*., 1996). This change in amino acid leads to a decrease in COMT activity by three to four fold, resulting in a hyperdopaminergic state. Prefrontal executive function is influenced by *COMT* enzyme activity, which affects the prefrontal dopamine activity, varying between individuals (Zhang *et al*., 2016). Individuals who are homozygous for Val (has two copies of this allele) have a higher COMT enzyme activity then those who are homozygous for Met, with heterozygous individuals (have both alleles) found to have intermediate *COMT* enzyme activity (Chen *et al*., 2004; Weinshilboum *et al.*, 1999).

In 2008 Hettema and colleagues reported that *COMT* contributes to genetic susceptibility amongst anxiety spectrum phenotypes, with rs4680 found to be significantly associated with anxiety phenotypes, specifically in females, supporting previous studies with similar findings (Hettma *et al*., 2008; McGrath *et al*., 2004; Kim *et al*., 2006). The Met allele of rs4680 has also been linked to an increased risk for PTSD, which has been found to interact with exposure to childhood trauma (Kolassa *et al*., 2010; Boscarina *et al*., 2011). Based on these findings, van Rooij and co-authors investigated the interaction between childhood trauma and the *COMT* rs460 genotype amongst 35 Met carriers and 35 control participants, using functional magnetic resonance imaging (MRI) in a response inhibition task (Go/NoGo) alongside self-report measures for childhood trauma exposure, resilience, PTSD and depression. Their findings

revealed an interaction between childhood trauma and rs4680 which lead to an increased risk for trauma-related psychopathology or resilience. Together with childhood trauma, the Met carriers had decreased hippocampal activation, while Val/Val carriers had an increase in hippocampal activation. Hippocampal activation was also positively correlated with trait resilience. Van Rooij *et al*. suggested that enhancement of resilience in individuals exposed to childhood trauma may be due to hippocampal recruitment during inhibition which improves the ability for one to use contextual information to guide behaviour (van Rooij *et al*., 2016). Due to the hippocampus playing an important role in both contextual learning and memory, it is believed to be involved in the regulation of inhibitory processes in light of contextual information, with dopamine which is regulated by *COMT*, suggested to be an essential neuromodulator of inhibitory processes (Chambers *et al*., 2009; Milad *et al*., 2007).

COMT SNP rs4633 is a synonymous polymorphism located on exon 3 of the MB-COMT transcript and exon 1 of the S-COMT transcript. Although this SNP does not lead to any change in amino acid sequence it has been found to influence *COMT* expression. The TT genotype encodes for low activity of the COMT enzyme which in turn leads to a decrease in *COMT* expression and dopamine degradation (Wu *et al*., 2014). In a recent study, rs4633 was reported was associated to high rates of PTSD amongst carriers of the CC genotype (Kwon *et al*., 2020).

Less studied *COMT* SNPs include rs737865 and rs2075507 (previously rs2097603), both of which are independent functional variants affecting *COMT* gene activity (Altinyazar *et al*., 2015). Located in intron 1, rs737865 is believed to affect the change in transcription rates by either alteration of a transcription factor binding site or adaption of alternative splicing processing (Bernegger *et al.*, 2018). SNP rs737865 has been identified to be a part of a three marker haplotype rs737865 (T)-rs4680 (G) - rs165599 (G) linked to panic and anxiety disorders (Wray *et al*., 2008).

Lastly, rs2075507, previously referred to as rs2097603, is located within the promoter region (P2) of *COMT* (Chen *et al*., 2004). In a 2004 study, Chen and colleagues reported on a significant effect of SNP rs2075507 in human lymphocytes, with genotype GG showing lower relative activity of *COMT* in comparison to genotype AA (Chen *et al*., 2004). The SNP rs2075507, found in the P2 promoter region, has been associated with inefficient prefrontal working memory responses, affecting attentional and executive functioning skills (Meyer-Lindenberg *et al*., 2008).

In studies based on South African population groups, a *COMT* haplotype; rs2020917-rs737869-rs6269-rs4633-rs9332377, was found to be associated with schizophrenia (Higgins, 2015). In another study, SNP rs4680 was investigated in South African Caucasian populations for OCD and associated risks. (Niehaus *et al.*, 2001; Lochner *et al*., 2005). Niehaus and colleagues recruited a total of 54 unrelated OCD patients and 54 controls from an Afrikaaner community, who were phenotyped using the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) and genotyped for rs4680 high or low activity alleles. Rs4680 was found to be significantly more common amongst OCD patients than expected (Niehaus *et al*., 2001).

This chapter describes the design and optimization of a multiplex assay to genotype several *COMT* SNPs with suggested association to anxiety, resilience or childhood trauma. A multiplex which can simultaneously genotype several *COMT* SNPs will allow for an efficient means to identify *COMT* haplotypes associated with anxiety, resilience and childhood trauma in a South African sample group. To our knowledge, the selected SNPs for this project as a possible functional haplotype has not been investigated as a potential risk factor for the anxiety, resilience or childhood trauma in a Southern African population group.

2.2 Methodology

2.2.1 Participant Recruitment

Prior to sample collection, ethical clearance for this project was approved by the Senate Research and Innovation Division Committee of the University of the Western Cape (BM17/6/21). A total of 263 participants volunteered for this study. Participants were recruited from undergraduate laboratory practical sessions at the UWC Life Science Building. Following a short overview of the research project by the researcher, volunteers completed a consent form (**Appendix I**) and were directed to an area within the laboratory for sample collection. All volunteers who provided a saliva sample signed a consent form thereby providing consent for their samples to be used in this study. In addition, these volunteers completed a genetic screening questionnaire (**Appendix II**) to capture all information required for accurate analysis of genotyping, which included ancestry, sex, age and ethnicity.

2.2.2 Sample collection

Volunteers had not smoked or had anything to eat or drink 30 minutes prior to saliva collection. To stimulate the production of epithelial cells, a salt-vinegar solution, consisting of 2% acetic

acid and 2% NaCl final concentration in a 1:1 ratio was given to each participant to swirl around in their mouths 5 minutes prior to saliva collection. All saliva samples were collected using a greiner tube with volumes ranging from 5ml to 15ml depending on the participants saliva production (Bio One, South Africa). Both saliva tubes and consent forms were handed in together, in order to de-identify the participant, by providing a numbered code on the tube which matched the respective consent form.

2.2.3 Sample preparation

Following saliva sample collection, an equal volume of storage buffer (under patent by the Forensic DNA Laboratory) was added to each saliva sample and stored at room temperature, in a restricted access room in the FDL at UWC.

2.2.4 Inclusion and Exclusion Criteria

All consent form information was captured into a Microsoft Excel sheet in order to capture demographic information. Samples which had incomplete accompanying consent forms and for which volunteers were not third generation South Africans were removed from the sample group, this was done to ensure that all volunteers in the sample group were considered South African. From the 263 saliva samples collected, 221 had completed forms, which was further reduced to 206 by eliminating those not third generation South African. This was done to ensure all data obtained is classified within South African context.

2.2.5 DNA Extraction

A modified salting out protocol (Miller *et al*., 1998) was used to extract DNA from all saliva samples. The protocol was modified to ensure maximum DNA yield and the steps involved are outlined below: A total of 500µl of each saliva sample was transferred to new, labelled Eppendorf tubes. A volume of 5µl 20mg/ml Proteinase K was added to each tube, followed by an overnight incubation of all tubes at 53°C and 40rpm.

Following overnight incubation, samples were cooled to room temperature and an equal volume of 4M NaCl was added to the tubes, which were vortexed at 135000 rpm. The samples were incubated on ice for 3 to 4 hours. Once incubation was complete, the samples were centrifuged at 135,000rpm for 10 minutes (until there was formation of a pellet). A volume of 800µl supernatant of each sample was transferred to new tubes containing equal volume ice

cold 100% ethanol (EtOH). All tubes were vortexed briefly and centrifuged at 135 000rpm for approximately 5 minutes.

The supernatant was discarded from each tube without dislodging the pellet and 100µl of 70% ice cold EtOH was added to the tubes to wash the pellet, followed by centrifugation at 135 000 rpm for 10 minutes.

The supernatant was discarded and the tubes were air dried in a laminar air flow for approximately one hour. Once all tubes were dry from excess EtOH, 50µl TE buffer (pH 8) was added to all tubes and rehydrated in a hot block at 55°C for 20 minutes, followed by brief vortex. All samples were stored at -20°C.

2.2.6 Normalization of DNA concentrations

All samples were quantified using the Nanodrop ND-2000 UV spectrophotometer (ThermoFisher Scientific, U.S) and was then made up to a standard concentration of 50-100ng/µl with TE, while purity between 1.7-1.8 (A_{260}/A_{280}) was ensured. All samples with a concentration below 50ng/µl or purity outside the range of 1.7-1.8 were discarded and re-extracted by salting out the original samples. Samples with high concentrations (>100ng/ul) were standardized, with the addition of TE buffer, to 50ng/ul.

2.2.7 SNP of interest selection

A total of six *COMT* SNPs of interest were selected for this study, using the NCBI (SNP) Database (https://www.ncbi.nlm.nih.gov/snp/, Accessed 12 June 2017) ensuring there has been published literature linking the SNPs to either anxiety, resilience or childhood trauma as discussed previously (Chapter 1). In addition to selecting SNPs linked to GAD, SNPs that were reported to be associated with other phenotypes but in African population groups was also considered as the required sample group was to be of African ancestry.

2.2.8 COMT SNP Flanking and SBE Primer Design

All primers were designed with the default parameters using the online software, BatchPrimer3 (https://probes.pw.usda.gov/batchprimer3/). Flanking and single base extension (SBE) primer outputs underwent a blast analysis using the NCBI Primer-BLAST tool (https://www.ncbi.nlm.nih.gov/tools/primer-blast/) to ensure the *COMT* SNP of interest was

identified for each respective primer pair and that the primers did not bind to any other region of the genome (**Appendix. III**). For the SBE primers needed for SNaPshot PCR, poly T tails were added to ensure no self-binding. All SNP primers are listed in **Tables 2.2.9** and **2.2.10**. Primers were synthesized by Integrated DNA Technology (IDT) and those <40bp were cleaned by high pressure liquid chromotograohy (HPLC). HPLC is recommended for oligos <40bp to enrich purity and condense the synthesized end product.

Table 2. 2 *COMT* SNP primer flanking sets

SNP*	Orientation	Len	Tm	GC%	Primer Seq	Product Size
1	FORWARD	20	59.97	45.00	AATTTGGCTATTGCCGTGTC	117
	REVERSE	20	60.15	45.00	CAAAGGGCATTTATCATGGG	
2	FORWARD	20	60.05	40.00	TTTTGGATTTTTCCAGCCAG	113
	REVERSE	20	60.62	60.00	AGTGTCTCACTGGGCTCTGC	
3	FORWARD	21	62.07	57.14	CTGACACGTCAGGCAACTGAG	130
	REVERSE	20	60.76	60.00	CAGTGCTCTGTGCTCCTCCT	
4	FORWARD	20	60.80	55.00	GCTGGAACGAGTTCATCCTG	149
	REVERSE	20	60.21	57.14	CTTCTGCTCGCAGTAGGTGTC	
5	FORWARD	21	61.20	61.90	GGGCCTACTGTGGCTACTCAG	146
	REVERSE	21	61.57	52.38	CATGCACACCTTGTCCTTCAC	
6	FORWARD	22	61.82	50.00	CTCATCACCATCGAGATCAACC	117
	REVERSE	20	60.08	50.00	CCCTTTTTCCAGGTCTGACA	

1:

rs2075507; 2: rs737865; 3: rs6269; 4: rs4633; 5: rs4818; 6: rs4680, TM: Primer melting temperature

Table 2. 3 *COMT* SNP primers for SNaPhot PCR

SNP	Primer sequence (extended)	Concentration (µM)	Tm	GC%	Polymorphism
1*	35 Poly T-TGTGAGTATGGGAAGGGGAA	55	60.31	50	G/A; G/T
2*	30 Poly T-GGATTTTTCCAGCCAGGG	48	60.39	55.56	A/C; A/T
3*	25 Poly T-GAACCTTGCCCCTCTGC	42	59.28	64.71	G/A
4	10 Poly T-CAGCGCATCCTGAACCA	27	60.53	58.82	T/C
5	20 Poly T-ACCAGGGGCGAGGCT	35	60.32	73.33	G/C
6	15 Poly T-GGTGGATTTCGCTGGC	31	58.58	62.5	G/A

1:

rs2075507; 2: rs737865; 3: rs6269; 4: rs4633; 5: rs4818; 6: rs4680, Tm: Primer melting temperature

*Primers were HPLC cleaned (<40bp)

2.2. 9 PCR amplification of COMT SNPs

Gradient PCR is an optimization assay used to determine the optimal annealing temperature (T_a) of primers by testing identical reactions with fixed primer concentrations, across a range of temperatures simultaneously. A series of gradient PCRs were run for each primer set to establish the most suitable annealing temperatures. Initially, the T_a for each primer set was calculated using the online NEB Tm calculator (https://tmcalculator.neb.com/#!/main). Two multiplex reactions were set up based on the T_M and product size (bp) for each primer pair, as seen in **Table 2.1**PCR reactions were set up as per the manufacturer's guidelines for the Q5 high fidelity polymerase (NEB, England) for both the gradient PCRs and multiplexes. Each reaction consisted of 5µl 10X buffer, 0.5µl dNTP's, 1,25µl of each forward and reverse primer (20µM), 0,25µl Taq (NEB, England), and 2µl DNA, made up to 25µl with RNAse free water.

Table 2. 4 Annealing temperatures for respective *COMT* SNP primer pairs, calculated using NEB Tm calculator, and the final annealing temperature after single-plex PCR optimization

	Primer	Tm by NEB (°C)	Annealing temperature (°C)
Multiplex 1	rs737865	57	61
	rs2075507	58	57-62
	rs4680	61	60-65
Multiplex 2	rs4633	62	60-65
	rs4818	63	61-66
	rs6269	65	61-66

2.2.10 Validation of PCR

PCR duplex reactions were validated by running all samples on a 3.5% agrose gel with X1 TBE at 55V for 3.5 hours. A 25bp marker (Bioline, Australia) was run with each sample set, alongside 2.5µl PCR product premixed with 1.5µl loading dye-GelRed mix. Gels were visualized under UV light with GelDoc

2.2.11 Post PCR Purification

Following amplification and validation, 15µl of sample PCR products were purified using 5 units of SAP (NEB, England) and 2 units of EXO I (NEB, England) as per manufacturer's instructions. The PCR products were purified individually before pooling, as recommended by

the manufacturer. Samples were mixed thoroughly and incubated at 37°C for one hour, followed by incubation at 75°C for 15 minutes to inactivate enzymes. Samples were pooled and stored at -20°C.

2.2.12 SNaPShot Extension

All SNaPshot PCR primers were made up to a final concentration of 0.2µM, as suggested by the manufacturer. A master mix was made up with all common components for each extension reaction. A final volume of 10µl for each reaction contained 5µl SNaPshot Multiplex Ready Reaction Mix (Applied Biosystems), 3µl purified and pooled PCR product, 1µl pooled primer mix and 1µl dH_2O. A negative and positive control, supplied by the manufacturer, was run with each reaction. SNaPshot PCR was run on a GeneAmp 2700 thermal cycler (Applied Biosystems), under the following conditions; 25 cycles of 96°C for 10 s, 50°C for 5 s and 60°C for 30 s, followed by a 4°C hold step. Samples were stored at -20°C.

2.2.13 Post Extension Purification

Post SNaPshot, all samples were purified with SAP. To each reaction tube, 1 U of SAP was added. Enzymes were deactivated by incubation of 75°C for 15 minutes, followed by a hold at 4°C for up to 24 hours prior to electrophoresis.

2.2.14 Genotyping

A total of 11 samples were used for the initial sample run before optimising the SNaPshot reaction. The first 11 samples produced results, with relative fluorescence units (rfu)>60. These samples were re-run with various adjustments to increase the rfu and reduce the noisy background.

Set 1: Increased *COMT* primer concentration from 0.2uM to 0.4uM in the initial PCR
Set 2: Increased SBE *COMT* primer concentration from 0.2uM to 0.4uM in the SNaPshot PCR
Set 3: Single SBE primer runs in triplicates

The first two sets were to understand if an increase in *COMT* primer concentration in either the multiplex PCR or SNaPshot PCR would yield better results. The third set was to ensure the base position of the SNP was consistent in each sample. The above samples were set up at the UWC FDL post PCR lab and then transported on ice to The University of Cape Town (UCT)

Human Genetics Laboratory for genotyping on an ABI Prism 3100 Genetic Analyzer (Applied Biosystems, USA). After each run, data files were analysed with GeneMapper V4.1 software (Applied Biosystems). The electropherogram produced for the three sets showed contamination in one of the SNaPshot reagents, and therefore was not valid., yet the allele peaks yielded for the increased SBE *COMT* primer concentration showed improved results with (rfu)>300.

2.3 Results

2.3.1 Sample characterization

Demographic information was captured for all study participants. Of the 206 individuals in the final sample, 70 were male and 136 were female, with an average age of ±20 years. The sample group consisted of mostly mixed ancestry (47.09%) and black African ancestry (34.47%) groups, with remaining 18.44% being Indian or not declared.

2.3.2 Gradient PCR analysis for the identification of optimal annealing temperatures

Temperature gradient PCRs for all six COMT SNP primers indicated the optimal annealing temperatures for each primer set. Most primer pairs had a broad range of acceptable Ta with the exception of rs2075507, which had an ideal Ta at 61°C (**Figure 2.1; 61A**). **Figures 2.1** to **2.3** shows the gradient PCR runs for each of the six primer sets, with the corresponding annealing temperatures for the gradient PCR. The first lane was loaded with a 25bp marker (M) (BioLine), followed by the negative water control (C).

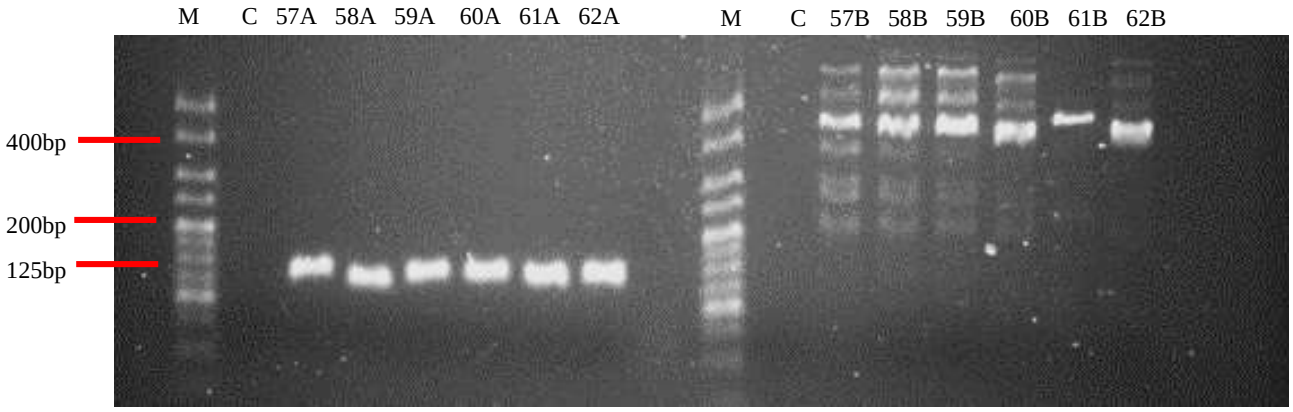

Figure 2. 1 Gradient PCR performed for primer set rs2075507 (left, A) and rs737865 (right, B), using annealing temperatures of 57-62°C. For primer set rs2075507, the amplified region is seen between 125-100bp, while primer set rs737865 has multiple bands amplified, excluding the expected size at 113bp. rs737865 amplifies a large region, including non-specific regions. Electrophoresis was run on a 3.5% agarose gel at 55V for approximately two hours with a 25bp marker (M).

Rs737865 was expected to show amplification at 113bp, as suggested by BatchPrimer3, however multiple products are seen being amplified in **Figure 2.1**, indicating the primer amplified other non-specific regions between 200-400bp, despite the BLAST result produced for the rs737865 primer pair which showed that the primer pair is expected to amplify MB-COMT on chromosome 22.

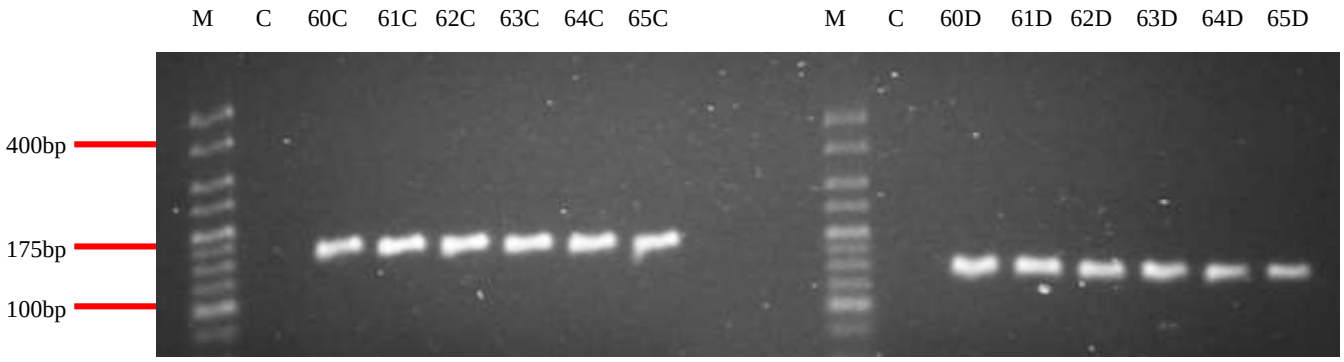

Figure 2. 2 Gradient PCR performed for primer set rs4633 (left, C) and rs4680 (right, D), using annealing temperatures of 60-65°C. For primer set rs4633, the amplified region is seen at approximately 150-175bp, while for primer set rs4680, the amplified region is seen between approximately 125-150bp. Electrophoresis was run on a 3.5% agarose gel at 55V for approximately two hours with a 25bp marker (M).

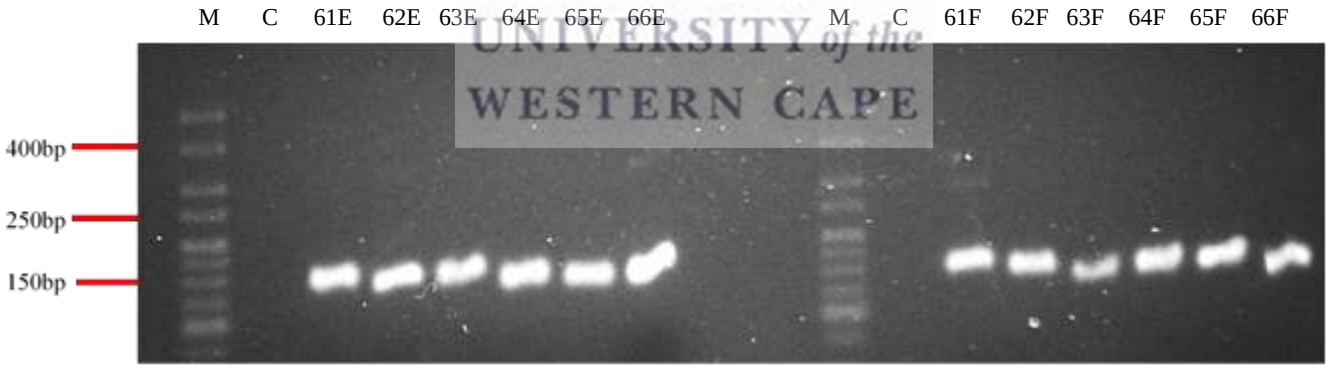

Figure 2. 3 Gradient PCR performed for primer set rs6269 (left, E) and rs4818 (right, F), using annealing temperatures of 61-66°C. For primer set rs6269, the amplified region is seen between approximately 125bp-150bp, while for primer set rs4818, the amplified region is seen at approximately 150bp. Electrophoresis was run on a 3.5% agarose gel at 55V for approximately two hours with a 25bp marker (M).

2.3.3 Creation of reference ladder

For most of the primer pairs, the product sizes are only a few bases apart and would therefore be difficult to discriminate between in a multiplex PCR reaction. For this reason, a reference ladder was created as this would assist in better discrimination of the different PCR products in a multiplex reaction. The reference ladder consisted of single template amplification for each SNP, run in a single lane on an agarose gel to create a ladder unique to the SNPs of interest. The products which appeared the clearest and brightest in the gradient PCR runs were selected, pooled and directly loaded with gelRED and loading dye, alongside the single templates which were selected for the ladder. **Figure 2.4** shows the reference ladder created with the corresponding selected samples. Due to the product sizes being similar for SNPs rs2075507 and rs4680, and the SNPsrs4633, rs4818 and rs6269, double and triple bands are seen in R1/R2 in the reference ladder, respectively. The creation of the reference ladder therefore would not aid in effectively discriminating between the different SNPs in a multiplex reaction.

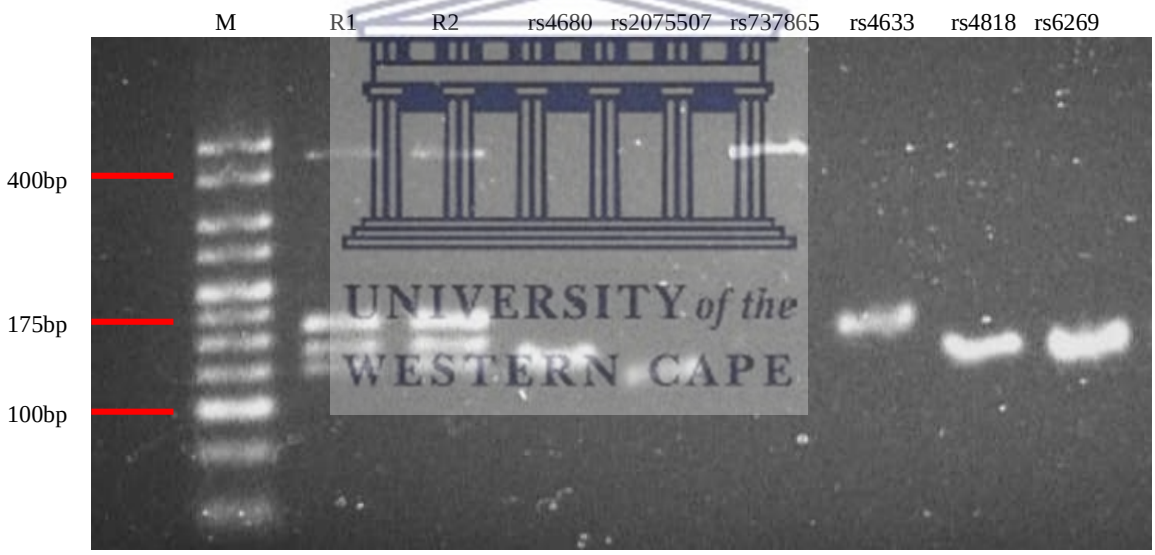

Figure 2. 4 The reference ladder created, seen in R1 and R2, with the corresponding single samples in lanes on sample 1. Each lane shows the respective *COMT* SNP amplified. Electrophoresis was run on a 3.5% agarose gel at 55V for approximately two hours with a 25bp marker (M).

2.3.4 Optimization of COMT SNP multiplexing

The multiplex reaction was initially made up of three primer sets (rs737865, rs4680 and rs2075507), with the same conditions as those stipulated for the gradient PCRs. According to results from the gradient PCR, each of these primer sets would amplify at a Ta 60-66°C. Due to primer set rs737865 showing best results for 61°C, this temperature was selected as the starting off Ta for the reaction. The reaction set up started off with two primers; rs4680 and rs2075507. Due to rs4680 and rs2075507 being the same in size, the products overlap, therefore the second band cannot be identified with certainty. Continuing, with the addition of the third primer set, rs737865 which has a distinct, larger band (400bp), amplification of product was not seen. In **Figure 2.5** the bands for the first three primer sets appear to be less visible. The poor visibility of the bands, and absence of the third band for rs737865 could be due to insufficient dNTPs in the reaction or too low primer concentrations.

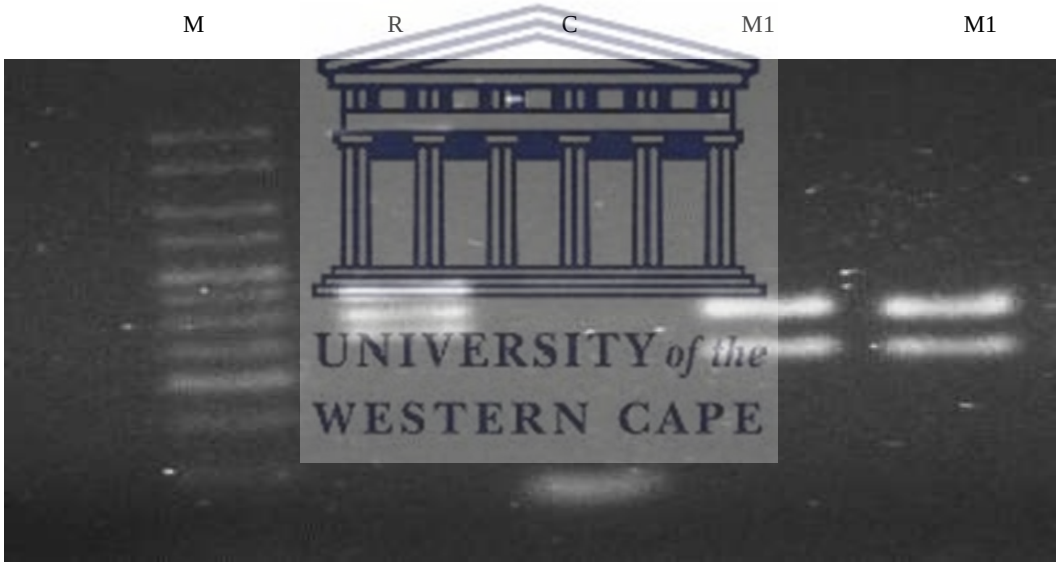

Figure 2. 5 Multiplex of primers, rs2075507, rs4680 and rs737865 on sample 10. Products were run in duplicate (M1 and M2) with s 25bp marker (M) and reference ladder (R).

2.3.5 Duplex selection for COMT SNPs

Due to the product size of each primer pair being less than 10 bp apart, validating PCR product on agarose gels was not feasible as it would be difficult to discriminate between PCR products of such similar size. As the multiplex of three COMT SNP primers did not provide the desired results, primer pairs were grouped together based on their annealing range to create three duplex reactions as follows; D1: rs4680 and rs4633, D2: rs2075507 and rs4818; and D3: rs737865 and rs6269, with the annealing temperature of D3 was increased to 67°C to decrease

the number of non-specific bands. **Figure 2.6** shows the amplification of 3 samples from the D1, D2 and D3 amplification. D3 showed the presence of additional non-specific bands. This is due to COMT primers annealing to a larger region than expected, resulting in amplification of multiple regions.

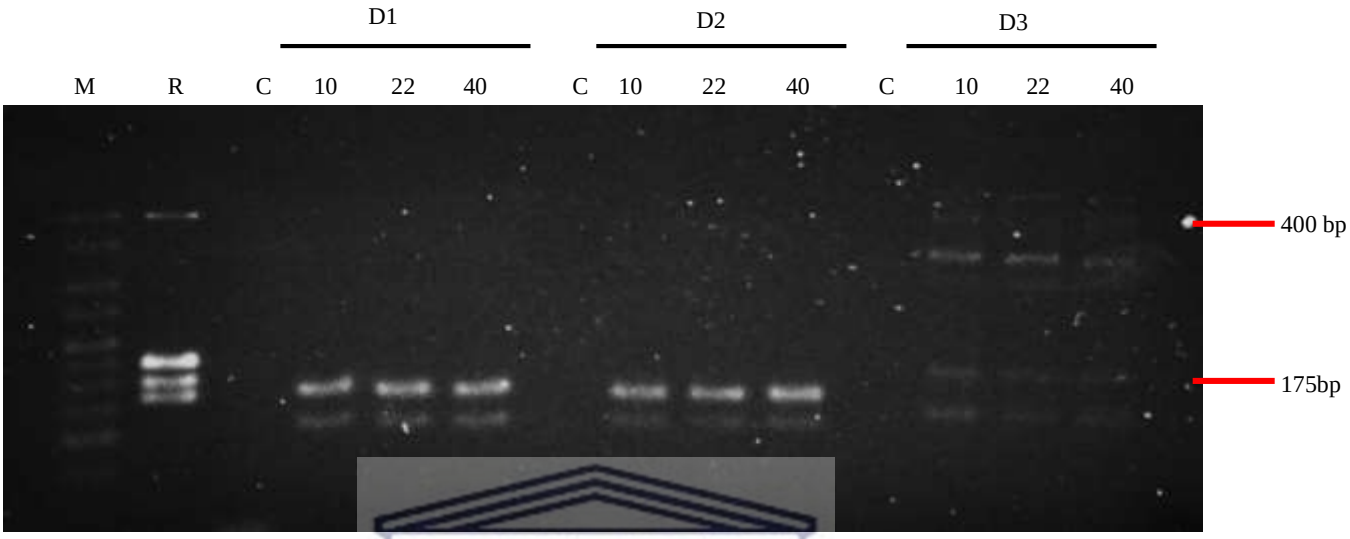

Figure 2. 6 D1, D2 and D3 sub set multiplex on samples 10, 22 and 40. Samples were loaded with a 25bp marker (M) (Bioline) and own reference ladder (R).

2.3.6 Genotyping of COMT SNPs

The subset of samples that were genotyped with the initial SNaPshot parameters produced a variety of inconsistent results. Samples 76, 78, 80, 83, 85 and 90 produced some peaks above 300 rfu, whereas sample 83 (**Figure 2.14**) all peaks above 300 rfu. These results suggest that the SNaPshot reactions needed additional optimisation such as increasing the primer or Snapshot concentration is required. The electopherograms of samples 10, 22, 40 and 60 did not show the expected base change for rs4633 of either T or C, indicative of a red or black peak. What was seen instead at that expected position was a single green peak (A allele) at the expected length. Sample 83 showed no observed base change for rs2075507. The identified fragment size (sz) for all SNPs shifted between 1-6 bases, which is a common occurrence. **Table 2.5** lists the expected and observed fragment length sizes for each SNP.

Table 2. 5 Identified polymorphisms for *COMT* SNPs, with expected fragment sizes differing for each of the respective SNPs

SNP	Expected FL*	Observed FL	Base/Change	Genotype
rs4633	27	33	T/C	C
rs4680	31	37	G/A	A
rs4818	35	40	G/C	C
rs6269	42	41	G/A	G
rs737865	48	44	A/C; A/T	A/G
rs2075507	55	51	G/A; G/T	A

*****Expected FL includes Poly-T tail, (FL)- fragment length

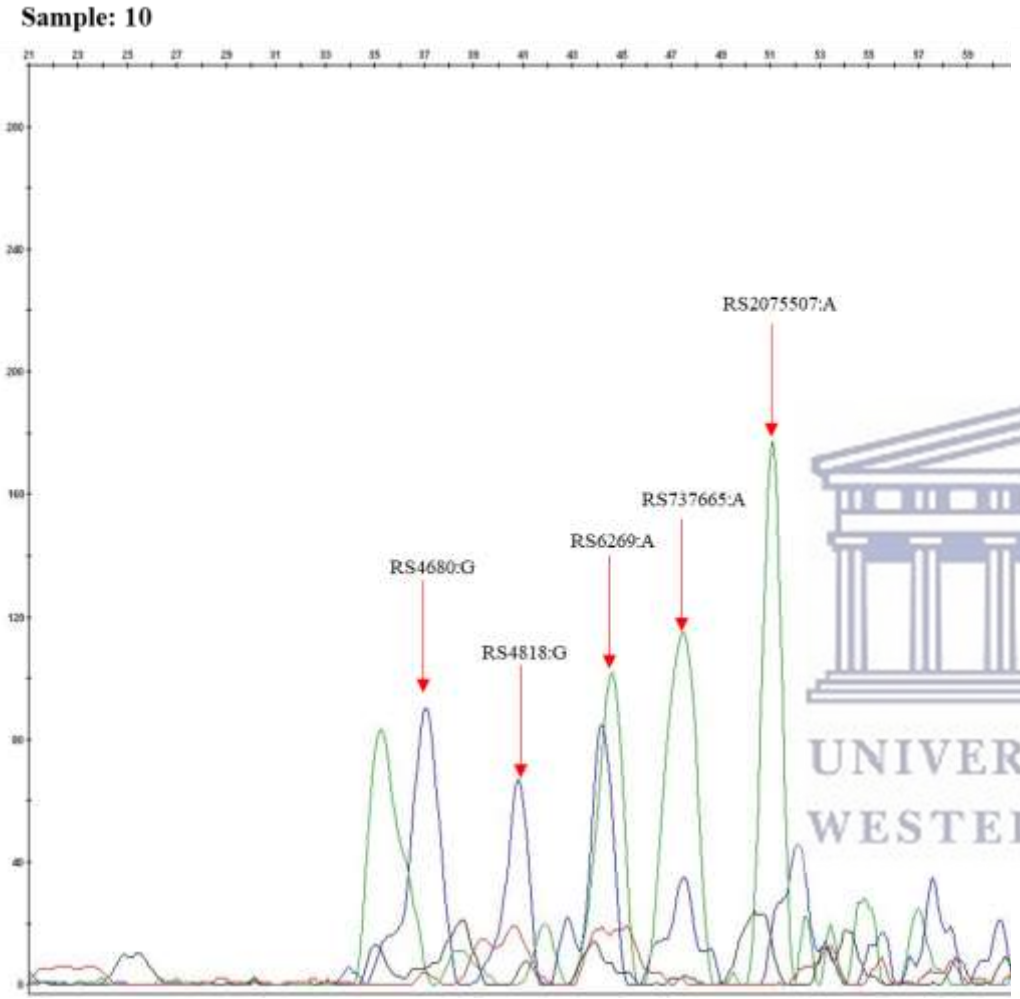

Figure 2. 8 An electopherogram on sample 10. No T/C peak was observed for rs4633, yet a peak of A was seen instead. All peaks >60 rfu.

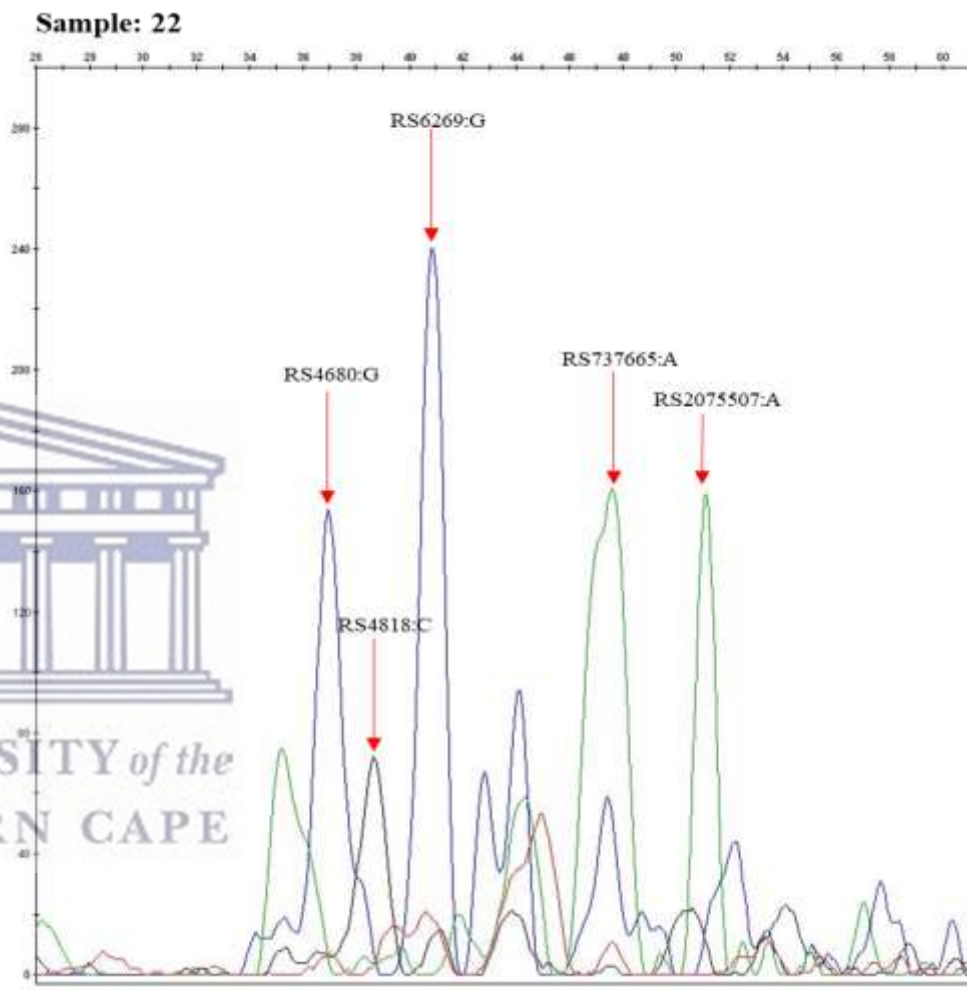

Figure 2. 7 An electopherogram on sample 22. No T/C peak was observed for rs4633, yet a peak of A was seen instead. All peaks >60 rfu

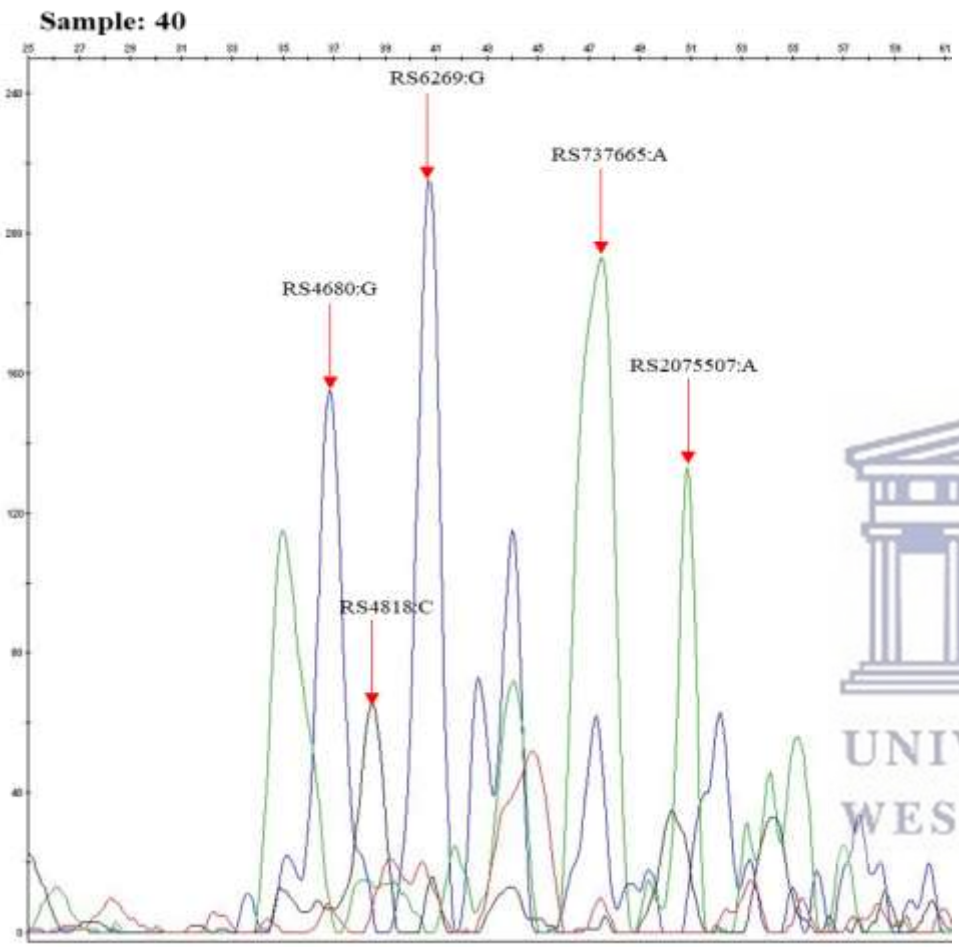

Figure 2. 10 An electopherogram on sample 40. No T/C peak was observed for rs4633, yet a peak of A was seen instead. All peaks >60 rfu

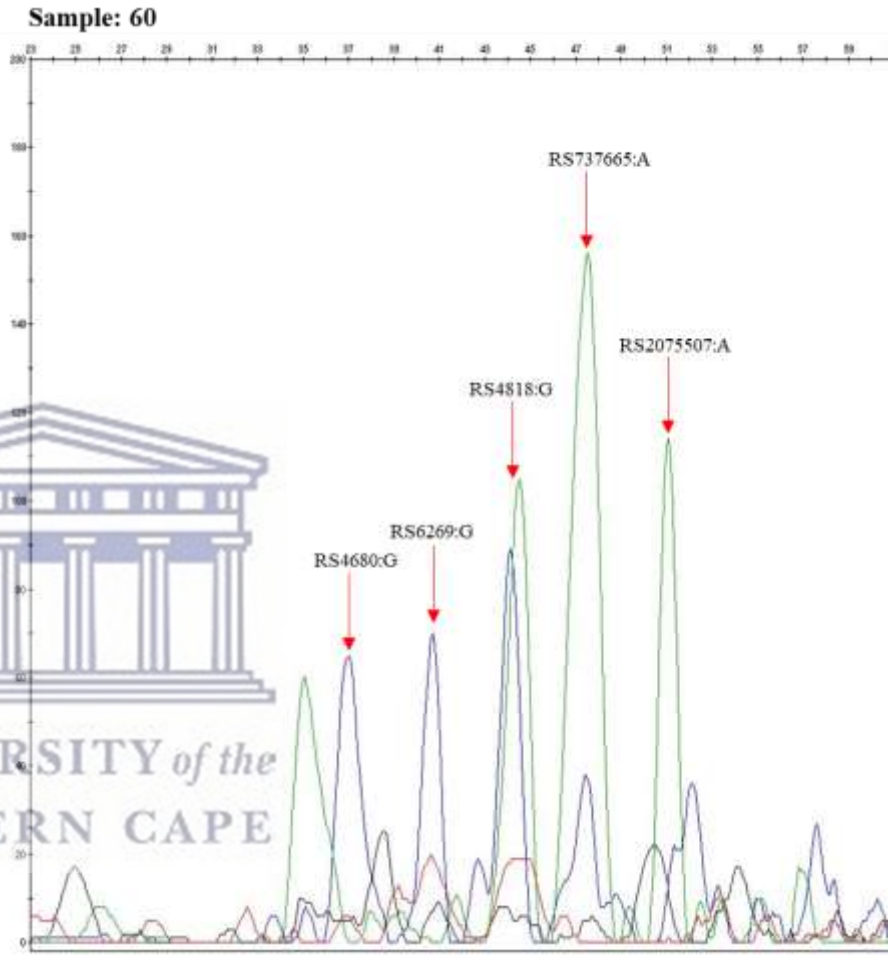

Figure 2. 9 An electopherogram on sample 60. No T/C peak was observed for rs4633, yet a peak of A was seen instead. All peaks >60 rfu

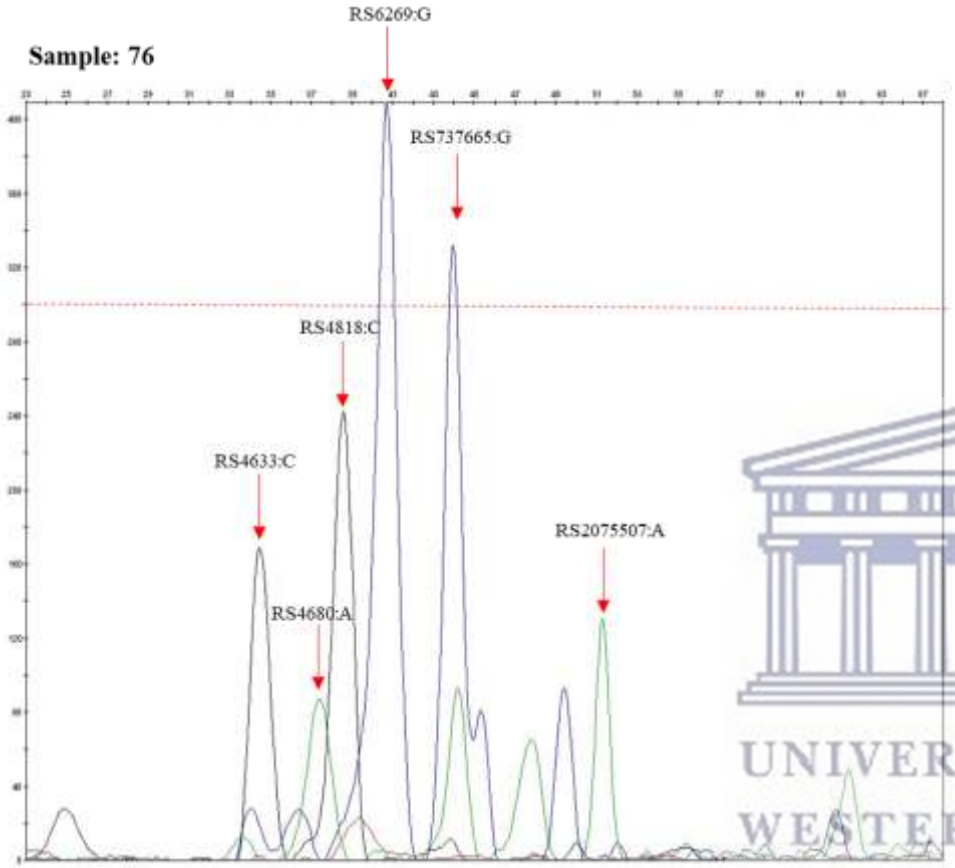

Figure 2. 12 An electopherogram on sample 76. rs62699 and rs737665 produced peaks <300 rfu, all other peaks >80 rfu.

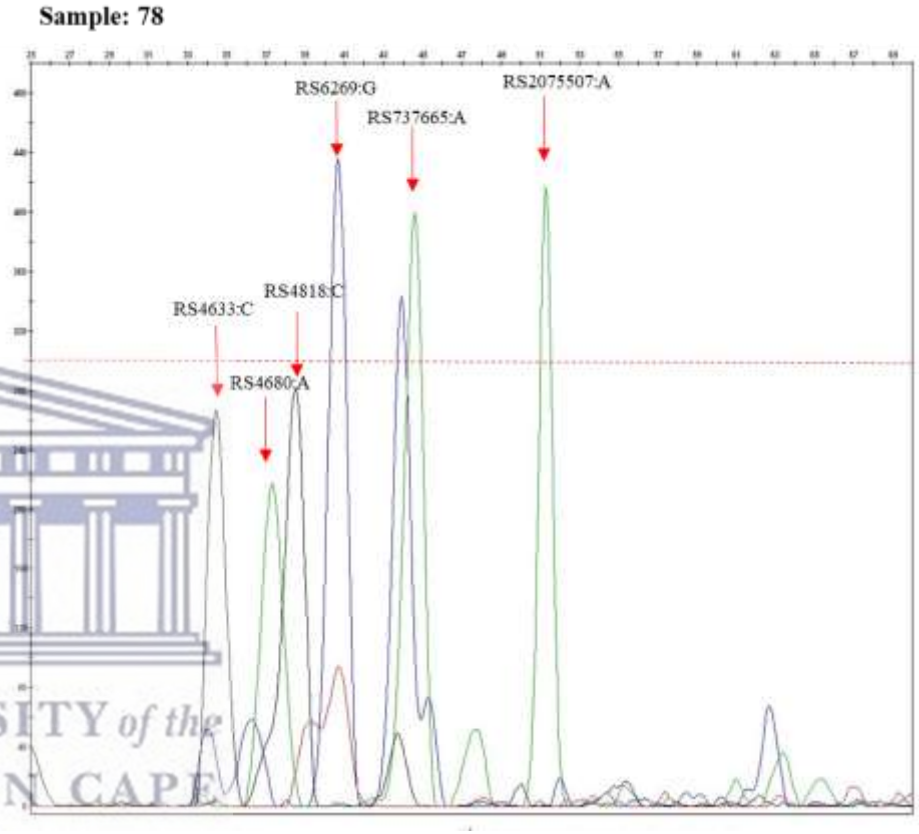

Figure 2. 11 An electopherogram on sample 78. rs62699, rs737665 and rs2075507 produced peaks <300 rfu, all other peaks >200 rfu.

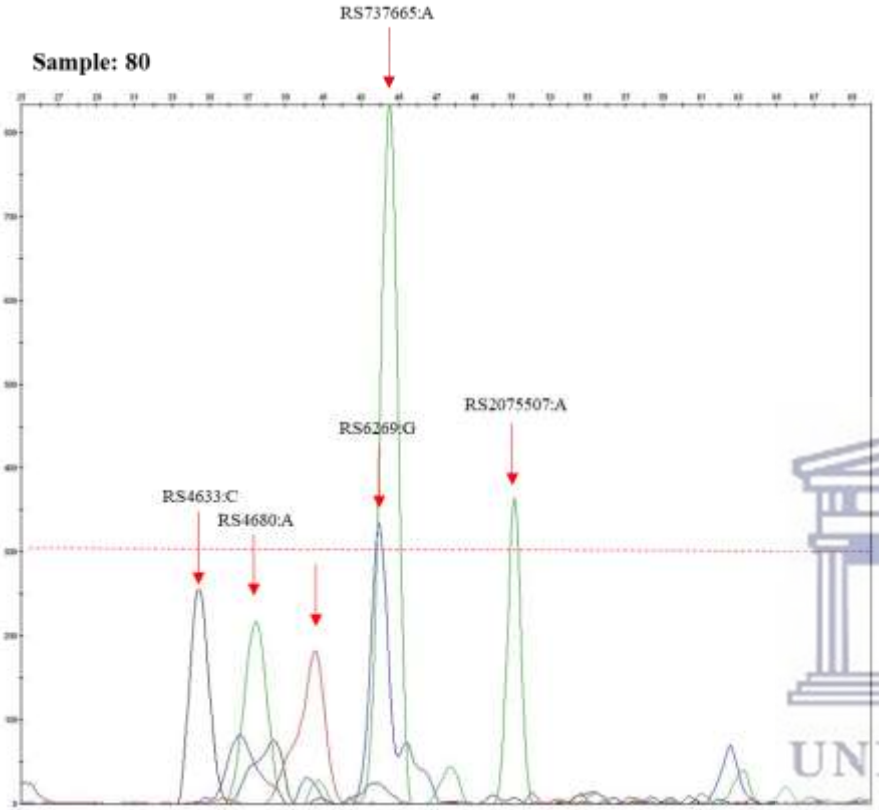

Figure 2. 14 An electopherogram on sample 80. rs62699, rs737665 and rs2075507 produced peaks >150 rfu, all other peaks >300 rfu.

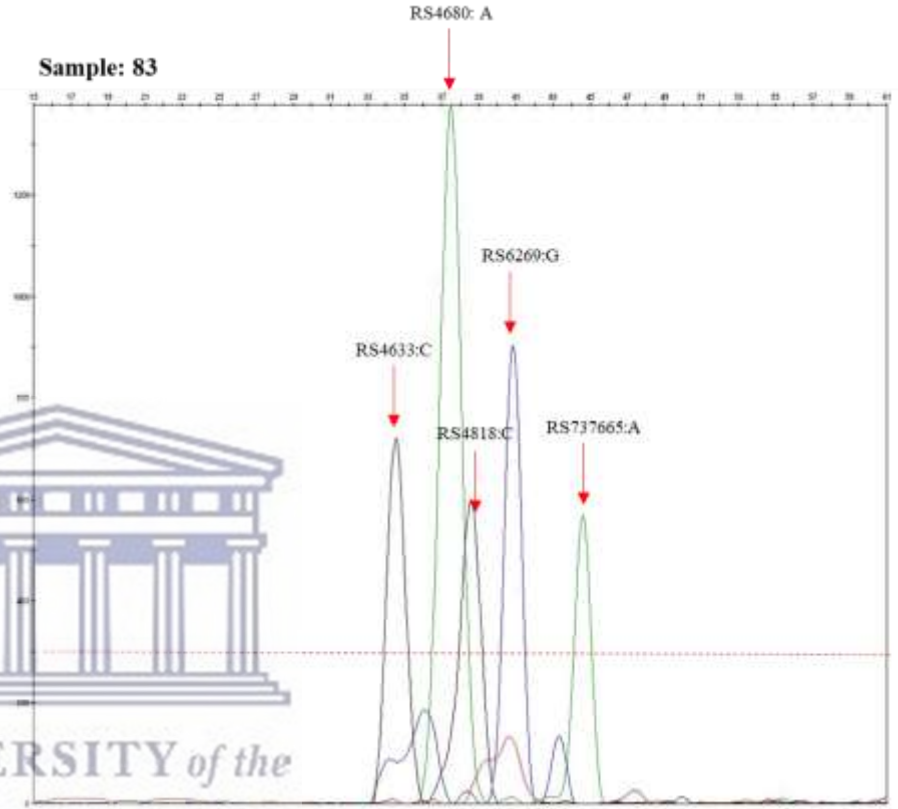

Figure 2. 13 An electopherogram on sample 83. All peaks >350 rfu.

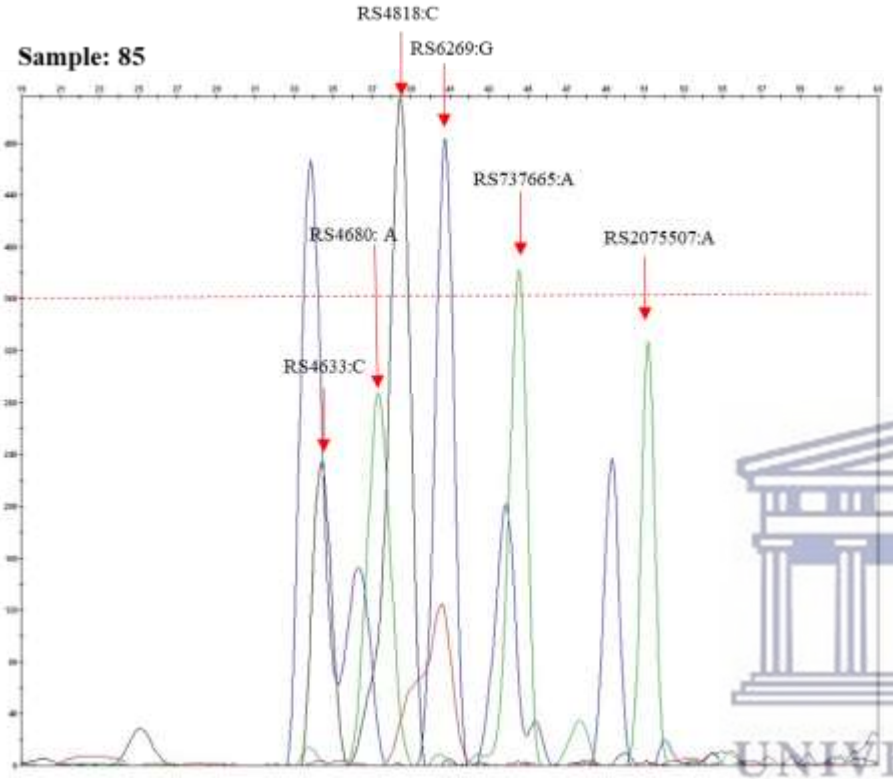

Figure 2. 16 An electopherogram on sample 85. rs4818, rs62699 and rs737665 produced peaks <300 rfu, all other peaks >200 rfu.

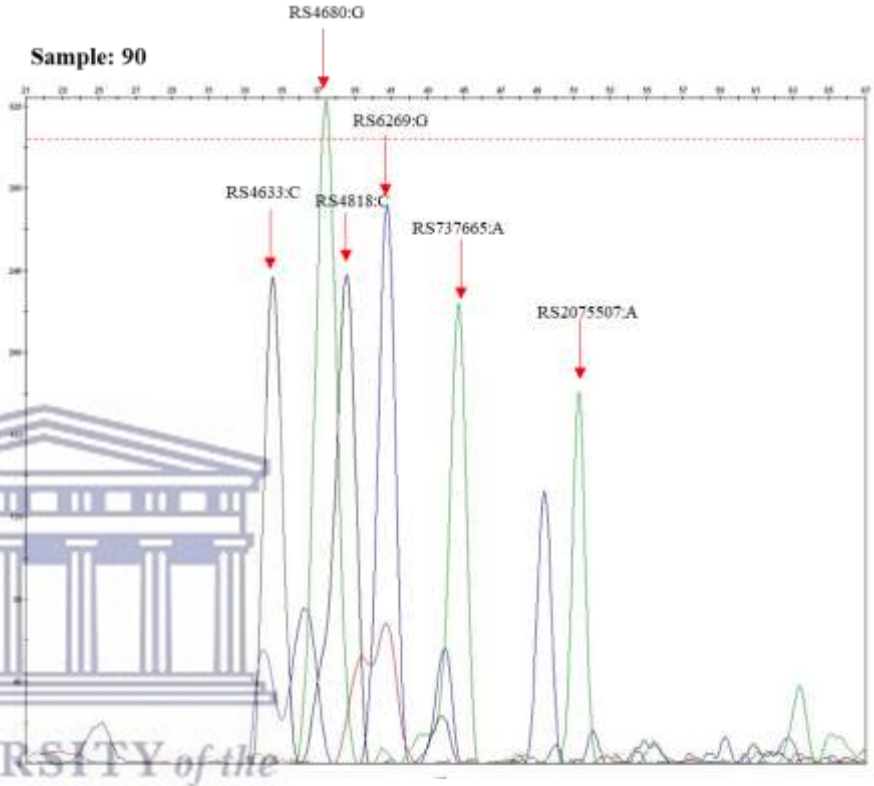

Figure 2. 15 An electopherogram on sample 90. rs4680 produced a peak <300 rfu, all other peaks >150 rfu.

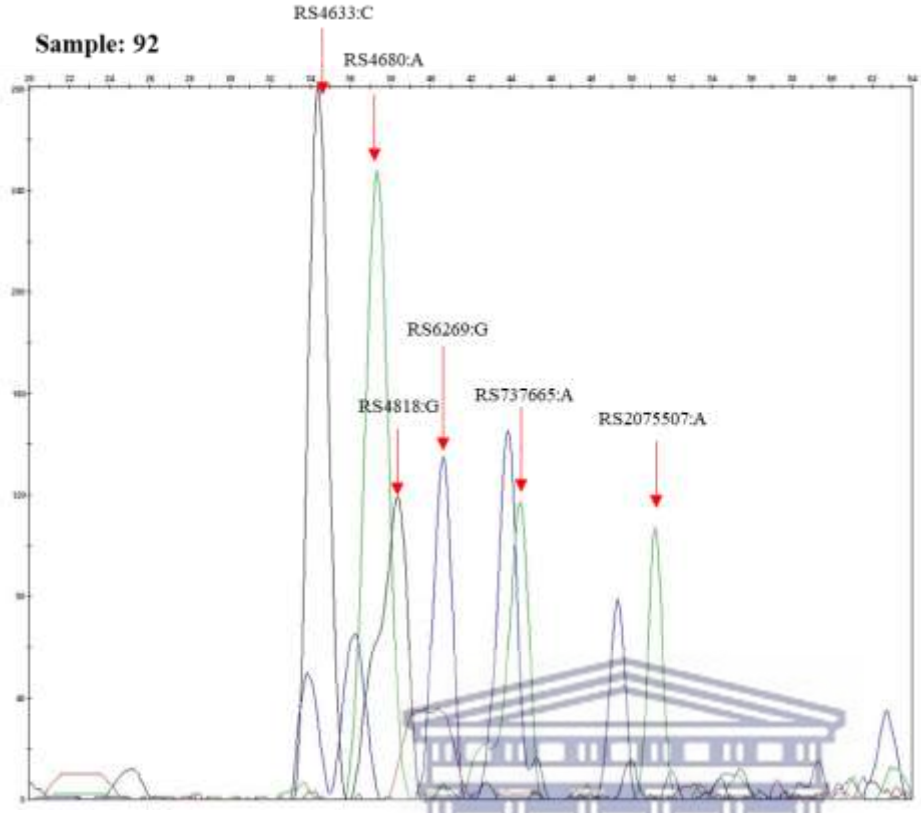

Figure 2. 17 An electopherogram on sample 92. All peaks >80 rfu.

The identified base changes for the sub set of samples genotyped were recorded at random (**Appendix III**), with their identified size and ancestral allele. For samples 10, 22, 40 and 60 no allele was identified for rs4633, as for sample 80 and rs4818; and sample 83 and rs2075507.

Based on the results for the 11 samples genotyped, the frequency of each allele change was calculated and compared to three population groups of similar African ancestry; namely Southwest US (ASW), Luhya in Webute, Kenya (LWK) and Yoruba in Ibadan (YRI). Due to the low sample number (11), this frequency distribution cannot be considered accurate in comparison to ASW, LWK and YRI, however was performed to provide a general understanding of allele frequency.

Table 2. 6 MAF for six SNP's of interest for the sample population group (SA), in comparison to African Ancestry in Southwest US (ASW), Luhya in Webuye, Kenya (LWK) and Yoruba in Ibadan, Nigeria (YRI)

SNP ID:	Polymorphism:	MAF[a]:	Genotype:	Population Group:			
				ASW	LWK	YRI	SA*
rs4633	C/T	T	CC	0.492	0.465	0.519	0.636
			CT	0.377	0.424	0.370	
			TT	0.131	0.111	0.111	
rs4680	G/A	A	GG	0.574	0.495	0.491	0.455
			GA	0.311	0434	0.407	0.546
			AA	0.115	0.071	0.102	
rs4818	C/G/T	G	CC	0.590	0.747	0.694	
			CG	0.393	0.222	0.269	0.727
			GG	0.016	0.030	0.037	0.181
rs6269	A/G	G	AA	0.410	0.424	0.426	0.182
			AG	0.508	0.404	0.435	0.818
			GG	0.082	0.172	0.139	
rs737865	A/G	G	AA	0.705	0.824	0.806	
			AG	0.279	0.165	0.176	0.909
			GG	0.016	0.012	0.019	0.091
rs2075507	G/A/T	G	GG	0.098	0.081	0.167	
			GA	0.525	0.424	0.417	0.909
			AA	0.377	0.495	0.417	

[a]MAF for African (AFR) sub-populations; ASW, LWK and YRI were taken from Ensembl SNP database (https://www.ensembl.org/index.html)

2.4 Discussion

2.4.1 The design and use of COMT SNP primers

Many association and linkage studies require the use of multiple SNPs to be genotyped simultaneously in large batch processing. Thus the aim of this chapter was to design and optimize a multiplex assay to genotype several *COMT* SNPs. Firstly, the primer design for the target region is an important initial step that will impact later downstream applications. BatchPrimer3 is able to design and generate multiple allele-specific primers simultaneously while increasing the specificity through an insertion of the 3-mismatch. A mismatch is intentionally introduced at the third position from the 3' end of each of the allele-specific primers to increase allelic specificity (You *et al*., 2008). To ensure designed primers will work effectively, there are a number of additional steps to be taken.

This includes checking the primer length, T_m, specificity and primer dimer formation. All of these mentioned steps were included in the primer design process, including the use of the BLAST function via NCBI to ensure the designed primers picked up the *COMT* gene on chromosome 22. BLAST results mirrored the primer reports from BatchPrimer3.

The single-plex and duplex reactions for *COMT* SNP primers worked for all but rs737865. Multiple regions on the template DNA was amplified ranging from 200-400bp, with no band observed at expected of 113bp. In practice, amplification of multiple regions other than the region of interest is due to non-specificity of primers. Despite the amplification of multiple regions with rs737865, post SNaPshot, the SNP for rs737865 was detected.

In the majority of SNP detection platforms, previous PCR amplification of the genomic region which flanks the SNP site of interest is required. BatchPrimer3 designs primer pairs which flank the SNP site of interest. SBE primers are specifically used in high-throughput detected platforms such as SNaPshot, as the SBE primer anneals immediately adjacent to the SNP of interest, extending it by one base using the fluorescently labelled ddNTP. BathPrimer3 designs primers for each orientation (forward and reverse), then selects the top candidates which meet the user-specified primer length range, followed by calculation of the T_m, GC and quality score for each candidate. The primers with the highest score is chosen (You *et al*., 2008). Due to SNaPshot being an extension assay with the use of a SBE primer which incorporates a dye, the primer designed for SNaPshot is more specific than the SNP flanking primer. It is therefore suggested that when designing primers using BatchPrimer3, the option for SNP flanking and SBE primer design is selected to ensure better specificity

To better understand how rs737865 yielded the expected result for SNaPshot yet unexpected amplification during PCR, the designed primer sequences were again checked for primer dimers using a multiple primer analyser (ThermoFisher Scientific) (https://www.thermofisher.com/za/en/home/brands/thermo-scientific/molecular-biology/molecular-biology-learning-center/molecular-biology-resource-library/thermo-scientific-web-tools/multiple-primer-analyzer.html) however the sensitivity for the parameters of primer detection was increased from “3” to “1”, where 3 indicates optimal detection and 1 indicates maximum sensitive detection. The results produced, showed the formation of three possible primer dimers which could account for the additional bands observed in **Figure 2.1.**

An additional BLAST was performed on the primer pair for rs737865, yet was set to BLAST against the full human genome, not limited to *COMT* on chromosome 22. The BLAST report showed that

aside from an expected product size of 113bp on Chromosome 22 for the *COMT* gene, additional regions of the genome could be flanked (**Appendix II**). This suggests that the primer pair designed for flank SNP rs737865 could have amplified other regions of the template DNA.

2.4.2. Optimization of multiplexing

Multiplex reactions, with some optimization, have a major advantage over singleplex reactions due to decreased time and cost efficiency, using less reagents such as dNTPs and enzymes. The large range of annealing temperatures for all six primers allowed for easier optimization during the multiplexing before SNaPshot, yet due to multiple SNPs having the same expected product size, it made validation on agarose gels difficult to resolve. One method often used for better resolving on agarose gels is with the use of higher percentage gels, however even with the use of 5% agarose gels, there is no way to confidently identify double templates at the same position.

Splitting the *COMT* SNP primers into duplex reactions, which were later pooled for SNaPshot appeared to be the best solution, as this still allowed for validation on agarose gels of two SNPs at a time. This method however proved to be time consuming, especially taking into consideration the running parameters on the 3.5% gels.

The primer design for all *COMT* SNPs led to many of the PCR products being of similar size and therefore challenging to discriminate between all on an agarose gel. The amplification of additional undesired template products, as well as the drop out in bands observed during the trial and error of multiplex mixes could be due to the way in which the *COMT* primers interact with one another under certain, unknown conditions. In theory, multiple target DNA regions can be amplified simultaneously in a multiplex PCR, however there are a number of issues which commonly occur that hinder this reaction, which include primer-primer interactions, primer-PCR product interactions; and formation of inhibitory secondary structures and thermodynamically favourable side products (Ozturk *et al.*, 2017). PCR products have different melting temperatures, which is dependent on their GC content length and sequence (Ririe *et al*., 1997). Due to this, the amplification of the *COMT* SNP region in the first PCR step was an important step to validate and optimize to later ensure genotyping via SNaPShot would be successful.

Majority of studies which focus primarily on the enzyme activity of *COMT* have investigated rs4680, with numerous reports of the Valine allele on rs4680 encoding for an enzyme which has high stability in a temperature range of 37-56°C (Scanlon *et al*., 1979; Lotta *et al*., 1995). It is these same studies which also report a difference between Valine and Methionine enzyme activity after the use of various

testing temperatures for enzyme stability. In addition to these authors, Spielmann *et al*., (1981) suggested the high variations amongst temperatures could be due to other SNPs indirectly affecting the activity of rs4680 (Spielman *et al*., 1981). Although there is much research which suggests other *COMT* SNPs, specifically rs737865 and rs165599 affect *COMT* expression, in schizophrenia studies, it has not yet been observed if any other *COMT* SNPs besides rs4680 affect enzyme activity (Shiftman *et al*., 2002).

2.4.3 The use of SNapshot as an analysis method for SNP identification

Although expensive, SNaPshot is an effective, highly accurate and reliable method of high throughput genotyping, serving as a more adjustable and reproducible method (Quintáns, *et al*., 2004). Although the second batch of samples for SNaPshot showed profiles which indicated contamination of either *COMT* primers or SNaPshot reagents, the initial "pilot" run on the randomized set of 11 templates produced results using the starting parameters, proving that little optimization would have been required. Genotyping was therefore limited to the 11 samples due to time constraints.

Significant noise is evident in the electropherograms, which may be due to a number of contributing factors which include; low purity DNA, low DNA concentration, inadequate primers, inefficient primer annealing or multiple priming sites. The fragment shift observed during capillary electrophoresis is a common occurrence, often due to the length, sequence and dye that is used for the labelled ddNTPs. Additionally, the extended products are altered causing the fragments to drift further from the expected size, which is due to the electrochemical nature of the assay (Quintains *et al*., 2004). As mentioned by Quintains *et al*., (2004), smaller fragments will drift further, which is seen in all 11 samples.

Since the introduction of SNaPshot, there have been a number of other assays which has been introduced, tested and used for SNP genotyping, one such assay is High Resolution Melt (HRM). This assay is based on a mutation scanning technique through the release of intercalating dye during denaturation which causes a progressive change in fluorescence. HRM can be performed in a tube, with only the addition of saturating intercalating dye and a high resolution melting required following PCR amplification (Witter *et al*., 2003).

As mentioned previously due to PCR products having different melting temperatures, HRM is a well suited genotyping method for SNP identification as the theoretical melting temperatures (T_m) can be calculated. Previous HRM related studies have observed that majority of homozygous sequence

polymorphisms produce a T_m shift in comparison to the wild type, while heterozygous polymorphisms cannot be identified by T_m but rather by the way in which the melting curve profile is produced (Palais *et al*., 2005; Graham *et al*., 2005). HRM has several advantages over SNaPshot, therefore future studies should consider using alternative methods to SNaPshot.

2.4.4 COMT SNP allele distribution

The absence of a full sample data set does not allow for any observation of possible *COMT* haplotypes or accurate allele distribution. Based on the just the 11 samples which were genotyped, serving as a "pilot" run, SNPs rs4818, rs629 and rs737865 included heterozygous genotypes. The observed COMT haplotype, based on the frequencies of the variants on the 11 samples were C-A-C-G-A-A/rs4633-rs4680-rs6269-rs737865-rs2075505.

Haplotype analysis has previously revealed possible protective COMT haplotype of G-A-A-A/rs2097603-rs737865-rs4680-rs165599 by Funke and colleagues (2005), which was under represented in the sample group (Funke *et al*, 2005). This haplotype was the opposite of that identified by Shifman *et al.*, (2004) which had reported an A-G-G-G haplotype, both of which were representative of psychotic disorders (Shifman *et al*., 2004).

A 2018 study on PTSD in traumatized Chinese, carried out genotyping on several SNPs of three dopaminergic genes, of which *COMT*; rs6269, rs4633, rs4818 and rs4680 were included. The authors reported the *COMT*, together with SNP rs1800497 of the dopamine receptor 2 (DRD2) affected the *COMT* enzyme activity at a protein to protein and DA level. They concluded with suggesting that the genotype combinations of the *COMT* and DRD2 SNPs to indicate a potential origin for DA homeostasis abnormalities in PTSD development (Zhang *et al*., 2018).

This component of the study presents a number of limitations, which halts valuable analysis. The genotyping of the full sample set would have allowed for statistical analysis for a better understanding of *COMT* SNP frequency and distribution. Despite this, the presented workflow serves as a basis for further, more detailed future work on the several *COMT* SNPs presented which is to include completed genotyping.

2.5 Conclusion

The main aim of this chapter was to design and optimize a multiplex assay in order to genotype several *COMT* SNPs, which proved to be partly successful. The design and optimization of a

multiplex for a total of six *COMT* SNPs was in this instance unable to be resolved, which lead to the use of duplex PCR reactions instead which proved to be successful. Perhaps more careful and detailed primer design, along with more vigorous PCR optimization would have led to successful multiplexing of all six *COMT* SNPs. The use of duplex PCR reactions instead, is sometimes a more efficient and reliable method for ensuring all SNPs of interest are amplified without the complexity of more than two primer pairs interacting with one another. However, this process may be more time consuming when working with large sample numbers, as the pooling of products prior to SNaPshot is an elaborate process.

The initial set up and run of SNaPshot using the SBE primers designed in BatchPrimer3 yielded results. The primer design, multiplex optimization and SNaPshot conditions used show good starting parameters that can be utilized and further improved through vigorous optimization. However, the use of SNaPshot for genotyping may have proved to be an efficient and sensitive assay, yet is an expensive method. Although previously temperature resolution was limited and not as well understood, which made this method of SNP genotyping rather inaccurate and unreliable, over recent years the introduction of new techniques, assays and dyes have made it a reliable SNP genotyping method with a growing and continued interest.

With only a total of six *COMT* SNPs of interest selected, this limits the reach of work done in context of the genetic link to anxiety, resilience and childhood trauma. A comprehensive and more in depth meta-analysis to identify not just one but a multitude of genes and possible variants associated to at least one of the functional behavioural categories will provide a better overview for possible haplotypes. The lack of studies which focus on South African population also warrants for further investigation in such populations for haplotype identification.

CHAPTER 3

THE RELATIONSHIP BETWEEN RESILIENCE, ANXIETY AND CHILDHOOD TRAUMA

3.1 Introduction

Psychological assessment for psychiatric disorders such as anxiety, as well as behavioural traits, contributes to the understanding of individual characteristics and capabilities which is obtained through a variety of measures with specific purpose for evaluation (Groth-Marnat, 2009). Self-report measures are one form of psychological assessment widely used for anxiety, resilience and childhood trauma.

3.1.1 Scale of Protective Factors

The Scale of Protective Factors (SPF-24) was originally developed by Ponce-Garcia *et al*., (2015) as a 35 item self-report measure for resilience, which was reduced to 24 items after exploratory factor analysis. Ponce-Garcia and co-authors (2015) tested 942 students from two institutions. Clinical criteria was used to identify participants exposed to violent trauma that had scored low, moderate or high on already established resilience scales. Results showed the low resilient group scored lower on all subscales of the SPF-24 (Ponce-Garcia *et al*., 2015). The SPF scale focuses primarily on contributing factors which form a buffer between the individual who has experienced some form of trauma, followed by stress and disruption. In total the scale includes two social interpersonal and two cognitive individual protective factors, while measuring resilience levels in young adolescents (ages 18-25) across levels of risk and socio-economic groups, ethnic groups and racial minorities (Madewell *et al*., 2019). It differs from other resilience scales as it is the only scale known to assess a broader range of protective factors, being the only one to include the assessing of social protective factors (Ponce-Garcia *et al*., 2015).

The development of the SPF was based on assessing protective factors which determine resilience, namely the social-interpersonal factor and cognitive-individual factor (Reich *et al.,* 2010). The development of the social-interpersonal factor was created using 11 items which reflect social support, confirmed by research which reported family and peer support as a contribution to resilience. Due to a variety of researchers which also reported social skills to contribute to resilience, a second sub-scale within the social-interpersonal factor was developed which consisted of eight items that

assesses social skills (Cohen *et al*., 2011; Jain and Cohen, 2013; Mastens and Coatsworth, 1998). The third sub scale, the cognitive-individual factor, was developed to include eight items which assesses prioritizing and planning behaviour, as previous research has shown that aspects of self-regulation contribute to resilience (Gardner *et al.,* 2008; Wong, 2008). A fourth sub scale, consisting of eight items was added under the cognitive-individual factor, which assesses the confidence in an individual's ability to accomplish goals and succeed The addition of this sub scale is supported by literature which shows that confidence and goal setting may predict positive outcomes in context of resilience (Carver, 1998; Nota *et al*., 2004).

The SPF therefore comprises of a total of four sub-scales used as an indication of strengths and deficits which contribute to an individual's resilience namely social skills and social support which are grouped as social, goal efficiency and planning, and prioritizing behaviour which are grouped as cognitive (Ponce-Garcia *et al.,* 2015). Scoring higher in either the social or cognitive sub scales indicate resilience in the respective category (Madewell *et al*., 2019). The understanding of resilience in young adolescents suggests that multiple domains work simultaneously, such as social and cognitive, which acts as a buffer for individuals in stressful situations which in turn lead to resilience (Reich *et al.,* 2010).

3.1.2 Brief Resilience Scale

The Brief Resilience Scale (BRS) was developed in 2008 by Smith and colleagues, with the purpose of assessing an individual's ability to "bounce back" or recover from stressful events, and instead thrive in adversity. Smith *et al*., (2008) examined four sample groups from New Mexico, which included students and chronic pain patients, finding that the BRS is a reliable measure for assessing resilience through 6 items on a 5 point Likert scale (Smith *et al.,* 2008). The BRS is a short 6 item self-report measure which has been validated in seven languages, namely, English, Dutch, Portuguese, Malaysian, Spanish, German and Chinese. Of the six items on the BRS, three are positively worded and the other three are negatively worded items, with the development and design of the scale based on protective factors such as social support (Amat *et al.,* 2014).

The BRS has been described as a reliable unitary construct, with it being linked to social relations, coping, personal characteristics and health. It has also been negatively associated with anxiety and physical symptoms (Salisu and Hashim, 2017). Smith and colleagues (2008) have suggested that the BRS may be of valuable use in behavioural research, highlighting that previous resilience measures

focus on personal characteristics of an individual that lead to positive adaption and not on resilience itself (Smith *et al.*, 2008).

3.1.3 Adult Resilience Measure

The Resilience Research Centre Adult Resilience Measure (RRC-ARM) is a 28 item self-report measure adapted from the Child and Youth Resilience Measure (CYRM) for adults as a means to assess the areas of resilience. It was developed across 14 international study sites, with the purpose to mirror the 1979 ecological model of development by Bronfenbrenner (Ungar and Liebenberg, 2013). The scale consists of three sub scales which represent the broader categories of resilience, namely; "individual"; "personal relationship with key individuals" and "context/sense of belonging". The individual sub dimension is aimed to evaluate self-resilience in key areas of one's personal wellbeing, while relationships with key individuals evaluates the extent of interaction with those closest, and lastly context evaluates other areas of daily life. Each sub-dimension can further be divided into clusters, which is comprised of areas of protective factors. The sub dimension "individual" is divided into three clusters: personal, and social skills and peer support. The sub dimensions "relationship with key individuals" is divided into two clusters: physical and psychological caregiving. The last sub dimension "context" is divided into three clusters: spiritual, educational and cultural (Ungar & Liebenberg, 2013). The scale focuses on both intra- and interpersonal protective factors that promote adaption in the face of adversity. The key factors which the scale was designed and developed upon, contribute to highly resilient individuals (Friborg, *et al*., 2003).

3.1.4 Anxiety disorders in South Africa

Grillion defined anxiety as a "state of heightened vigilance", linked to a general increase in the sensory sensitivity of an individual due to some form of either conflict or uncertainty (Grillion, 2002). In South Africa, anxiety disorders are the most common form of mental illness, affecting 1 in 5 individuals (South African Depression and Anxiety Group, 2018). In a large scale population based study by the South African Stress and Health (SASH) group, it was reported that anxiety was the most prevalent lifetime disorder amongst South Africans, with the highest rates reported in the Western Cape (Herman *et al*., 2009). With anxiety being one of the most well studied mental disorders, there are numerous well validated self-report measures used in both clinical and research settings.

Seedat and colleagues (2009) reported further on results from the SASH study, concluding that relationship problems, coupled with recent negative life events serve as markers for lifetime disorders,

such as anxiety. Interestingly, they reported that early adverse childhood experiences were associated with mood disorders rather than anxiety disorders (Seedat *et al*., 2009). The findings by Seedat and colleagues highlighted the importance of considering all contributing factors when investigating functional behavioural categories; namely anxiety, resilience and childhood trauma

3.1.5 Social Anxiety Questionnaire 30 for Adults

The Social Anxiety Questionnaire for Adults (SAQ-A30) was developed collectively between 18 Latin American countries, Spain and Portugal, with 58, 000 general participants and over 1,000 social phobic patients. Over a period of 6 years 1,000 participants recorded more than 10,000 social scenarios. Social anxiety experts selected scenarios for the initial analysis, reducing the number of scenarios to 512, which was used for the development of the SAQ. Following this study, the first version of SAQ-A was composed, which underwent a number of statistical and clinical analysis to produce the final version, SAQ-A30 (Caballo *et al.*, 2010).

The SAQ-A30 is used to measure both specific and/or generalized social anxiety in adolescents 18 years and older, in general and clinical population groups, and has become a useful measure for identifying individuals which experience generalized social anxiety (Caballo *et al.*, 2012). It consists of 30 items, with a 5 point Likert scale for the participant to communicate the level of discomfort, stress or nervousness in a number of social situations (Cabello *et al.*, 2010). A total of five dimensions of social anxiety are assessed in the SAQ-30, namely; speaking in public/talking with people in authority, interactions with the opposite sex, assertive expression of annoyance, disgust or displeasure, criticism/embarrassment and interactions with strangers. Caballo *et al.* (2010, 2012, 2013), reported the five dimension subscales to be reliable and stable in both clinical and communal samples, with cumulative variance of 40.80-54.39% and Cronbach's Alpha ranging from .88 to .93 (high) and .66 to .90 (moderate to high); concluding reliable consistency (Caballo *et al.*, 2010; 2012, 2013).

3.1.6 Spielberger State-Trait Inventory

Spielberger State-Trait Inventory (STAI) was developed in 1983 with the purpose of assessing levels of both state and trait anxiety in adults through 40 items using a 4 point likert scale (Spielberger *et al*., 1983). The scale is divided into two parts of 20 items each, part 1 (STAI for Y-1) addresses state related situations, while part 2 (STAI form Y-2) assesses trait related situations, with each respective section varying in wording of items and response instructions. The likert ranges from 1 to 4, with 1

being ‘almost never’ and 4 being ‘almost always’. A lower rating would therefore indicate low levels of anxiety, while a higher rating indicates a higher level of anxiety (Spielberger *et al*, 1983).

The STAI has been used in numerous studies to evaluate anxiety and has been translated in 48 languages. Data from both heterogeneous and psychiatric adult sample groups support internal consistency and validity of the STAI in over 3,300 studies, making it one of the most reliable measurements for anxiety amongst researchers and health professionals (Kabacoff *et al*., 1997; Stanley *et al*., 2001; Ladd and Gabrieli, 2015). According to Spielberger (1983), because anxiety is a one-dimensional construct, the higher the trait anxiety, the higher the state anxiety will be in various and specific threating situations (Spielberger, 1983). Supported by many studies which use the STAI, state and trait anxiety scores correlate which proves their association. A high state anxiety score will equal a high trait anxiety score, which supports theoretical suggestions that trait anxiety arises from state anxiety and the two co-exist (Julian, 2011). The splitting of the STAI scale into state and trait assists researchers to characterize anxiety “proneness” as a longstanding characteristic, making the trait section of the scale less responsive to change as compared to the state section (Julian, 2011).

3.1.7 Beck anxiety inventory

The Beck Anxiety Inventory (BAI) is used to measure the severity and level of anxiety for ages 17 to 80 years. Originally developed by Beck and colleagues (1998), a revised version was later published in 1993 with changed scoring. BAI consists of 21 items with a 3 point Likert scale and classifies levels of anxiety on raw scores as follows: minor anxiety (0-7), mild anxiety (8-15), moderate anxiety (16-25) and severe anxiety (30-63) (Beck *et al.*, 1998; Halfaker *et al.*, 2011). It has been widely used in mental health research in clinical settings, yet has been disputed by some researchers for its emphasis on psychophysiological symptoms which are linked to panic, making use of statements to be rated by the participant such as “fear of losing control” and “difficulty in breathing” (Muntingh *et al*., 2011). A number of studies have reported higher BAI scores in individuals with PD than those with GAD (Cox *et al*., 1996; Fydrich *et al.*, 1992; Leyfer *et al.*, 2006). Despite these findings, individuals with PD and other anxiety disorders have been found to score notably higher than those without anxiety (Kabacoff *et al*., 1997; Wetherell *et al*., 2005)

The BAI was originally designed for use in clinical samples, however due to the simplicity and conciseness of the scale it has been used in a range of research settings, which include non-clinical samples. It has also been reported to be a better measure of anxiety than the Spielberger STAI, therefore it is important to examine the scale in non-clinical populations (Creamer *et al*., 1995). One

study in particular investigated the properties of the BAI in comparison to the STAI, comparing scores from both scales, in a non-clinical population of 326 undergraduate university students. The authors reported the BAI to demonstrate good psychometric properties with a high level of internal consistency. In comparison to the STAI-Trait, low test-retest correlation was reported which suggested the BAI functions well as a measure of state anxiety. In addition, the BAI was found to have a stronger ability to differentiate anxiety from depression in comparison to the STAI (Creamer *et al*., 1995).

A South African study (2019) which evaluated the use of the BAI as a predication indicator for GAD among individuals seeking HIV testing in the Western Cape concluded that while BAI can be used to measure and screen GAD, it is likely to produce a number of false positives, suggesting that to determine true cases of GAD, BAI should be used in conjunction with other reputable self-report measures for anxiety, such as STAI (Saal *et al*., 2019)

3*.1.8 Adverse Childhood Experience*

The Adverse Childhood Experience questionnaire (ACE) is a short 10 item self-report measure which originated from the ACE study in 1988. The study included a total of 8,506 adults aged 19-92 years, with a diverse range of ancestry. The purpose of ACE is to detect adverse childhood experiences of abuse and neglect. Although the idea of ACE originated in 1988 by Feletti and co-authors, the research was continued by the Centres for Disease Control and Prevention and Kaiser Permanente, resulting in one of the largest epidemiological studies in the United States (Felitti *et al*., 1998). The study consisted of 17 000 adolescents whom completed the ACE questionnaire and physical examination in order to understand if an association between adverse childhood experiences and health issues, including physical and mental concerns, exists. Findings of the ACE study suggest that there is significant association between adverse childhood experiences and physical/mental illness later in life. It has been used to help identify those with high scores as having increased risk factors for health issues (Anda *et al.,* 2006).

3.1.9 Childhood Trauma Questionnaire

The Bernstein Childhood Trauma Questionnaire (CTQ), developed by Bernstein and Fink (1998) is a standardized self-report measure to address and measure the extent of five variations of childhood trauma which is identified as sub dimensions of the scale. These sub dimensions are emotional abuse, emotional neglect, physical abuse, physical neglect and sexual abuse (Bernstein and Fink, 1998; Villano *et al*., 2004). The CTQ is a retrospective scale used to detect any of the five areas of abuse in

children or adolescents and has been widely used in a number of diverse populations in studies which include the assessment of the impact of childhood trauma (Karos *et al.,* 2014, Hernandez *et al.,* 2013; Thombs *et al*., 2009) Initially the CTQ validation was determined amongst community and psychiatric groups which used several types of assessment methods for childhood trauma as a comparative measure. The CTQ was further validated in studies which made use of community, at risk and clinical samples (Thombs *et al.,* 2009). However, there has been some controversy over the validation of the scale as it has been shown that individuals struggle to differentiate between emotional and physical neglect, which leads to improper scoring and potentially unstable results for the two neglect subscales (Petersen *et al*., 2014).

In 2002, a South African based study by Lochner *et al.* investigated childhood trauma in 74 participants with OCD, 26 with OCD with trichotillomania (hair pulling) and 31 healthy controls, with the use of the CTQ questionnaire. It was reported that childhood trauma was much higher in OCD groups in comparison to healthy controls with OCD groups scoring higher on emotional neglect (Lochner *et al.,* 2002). These findings were consistent with other studies which suggest an association between anxiety-related disorders, such as OCD and childhood trauma (Kroksa *et al*., 2018; Norrholm and Ressler, 2009). Results suggested that early childhood trauma or stress serves as an indicator for higher risk of developing physical and mental health problems in adolescents which included anxiety disorders (Danese *et al*., 2012; Hughes *et al.,* 2017; Kalmakis *et al*., 2015). A recent study on 102 South Africans (2019), investigated the relationship between the behavioural inhibition (BIS) and activation system (BAS), with the quality of life in individuals that suffer from social anxiety disorders (SAD) with and without childhood trauma exposure. Results showed frequent childhood trauma exposure to be associated with greater SAD, it was further reported that healthy controls had lower anxiety levels and better quality of life than the group with SAD (Bruijnen *et al.,* 2019)

Investigating the relationships between resilience, anxiety and childhood trauma in a South African will allow for researchers and health professionals to better understand all three functional behavioural categories as a multidimensional construct. This will allow for the development of better interventions and strategies used in communities that are designed to assist in decreasing the risk of anxiety, reduce exposure to childhood trauma and implement support for those affected by adverse childhood conditions.

3.1.10 The Adverse Childhood Environment questionnaire

The Adverse Childhood Experiences (ACE) reports on traumatic events experienced during childhood and often has a significant impact on the emotional, physical and mental health of an individual later in life (Herzog and Schmahl, 2018). ACE's was first defined by Felitti and colleagues (1998) after the publication of data from their ACE Study, in collaboration with the Centres of Disease Control and Prevention (CDC) (Felitti *et al.,* 1998). With a total of 17,421 participants, the study aimed to identify the ten ACE's which showed a positive correlation to chronic mental disease in adolescents (Stevens, 2012). There are a total of ten ACEs which have been identified by researchers as risk factors for mental health in adolescents, these are; emotional, physical and sexual abuse; emotional and physical neglect; violent treatment towards ones mother, household substance abuse, household mental illness, parental separation, and having an incarcerated household member (Baglivio *et al.,* 2014).

This chapter serves to investigate the prevalence of anxiety, resilience and childhood trauma amongst the full sample group and between sexes through established self-report scales and respective normative data, while also investigating correlations between anxiety, resilience and childhood trauma in both the full sample and between sexes.

3.2 Methodology

3.2.1 Selection of self-report scales

A site-wide literature search was performed to establish the most commonly used scales in non-clinical populations to measure anxiety, resilience and childhood trauma on The National Centre for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov/), BioOne (https://bioone.org/) and Science Direct (https://www.sciencedirect.com/) databases. A list of possible scales was compiled for anxiety, resilience and childhood trauma, from which three scales were chosen for resilience and anxiety, and two for childhood trauma. The selection of these scales was based on a number of contributing factors which include reliability of the scale, population parameters, supported literature, and ease of access to both the scale and scoring manuals. The final selection of scales chosen are displayed in **Table 3.1**.

Table 3. 1 Functional behavioural categories of scales

Functional Category	Scale
Measure of resilient behaviour	Scale of Protective Factors (SPF 24)
	Brief Resilience Scale (BRS)
	Adult Resilience Measure (ARM)
Determination of prevalence of anxiety	Spielberger's Trait-State Anxiety Inventory (STAI)
	Beck Anxiety Inventory (BAI)
Determination of adverse environmental factors	Adverse Childhood Environment (ACE)
	Bernstein Childhood-trauma Questionnaire (CTQ)

3.2.2 Creation of questionnaire form

The scales selected were combined to create a single questionnaire on Google Forms, **Appendix IV**, to facilitate response from participants. The form was divided into four sections as follows: Section 1 participant information; Section 2 resilience scales; Section 3 anxiety scales; and Section 4 childhood trauma scales. These section categories were not available to the participants, in order to reduce bias or inconsistency in participant response. Included in Section 1, were questions relating to the use of medication and clinical diagnoses of mental illness by medical professionals, including anxiety disorders. Participants were also asked to report any disabilities.

3.2.3 Exclusion and inclusion of participants

Participants which had responses missing or incomplete sections which affected the scoring and analysis were contacted to complete the missing sections. All forms were then re-checked and any forms that could not be used due to missing responses were excluded from the final analysis. If they reported: any form of medical illness which required them to take prescribed medication, or clinical diagnoses of mental illness, including anxiety; they were still include. This was done to ensure the sample group is representative of a true university population. To understand population characteristics, the average age was calculated; and the racial group which the participant felt best described them.

3.2.4 Data capturing and scoring

All responses captured from the Google Form were exported into an Excel sheet for cleaning in preparation for scoring. All participants were de-identified by removing the names captured and replaced with the corresponding participant ID. Any duplicate entries, where a participant submitted responses for any section of the questionnaire twice, were removed, selecting the first responses for capturing of scores (**Appendix V**). Any incomplete responses were corrected using corrective scoring for the specific scale. This was done specifically for SPF which had responses missing for questions 4, 15, 16 and 17 for a total of four participants. Question 4 was in the dimension of social support, therefore the total score was divided by five instead of six, question 15 was in the dimension of planning and prioritizing behaviour, therefore the total score was again divided by five instead of six and questions 16 and 17 was in the dimension of social skills, therefore the total score was divided by four instead of six. For ACE, four participants had incomplete responses, these participants were removed from the ACE data as corrective scoring could not be applied to ACE due to the nature of response method being "yes" or :no". The responses captured were grouped per scale on new respective sheets in Excel, to compute the scoring per scale.

The scoring for the scales used was as follows; for the Scale of Protective Factors (SPF) (Likert 7 scale) mean scores were calculated per sub dimension (total of 4 dimensions), with a total of 6 questions per dimension as shown in **Table 3.2**. Overall scores range from 24-120, while sub dimension scores range from 6-30. Mean scores below 5 indicated a deficit in the protective factor (Ponce-Garcia *et al*., 2015). For participants 84, 156, 235 and 238, responses were missing for questions 4 (social support), 15 (planning/prioritizing behaviour); and questions 16 and 17 (social skills), to which corrective scoring was applied. Each of the dimensions for the SPF is made up of 6 questions, therefore for the above participants the missing question reduced the respective dimension sum. For social support and planning/prioritizing behaviour the total to be divided by was 5 and for social skills it was 4.

Table 3. 2 The Scale of Protective Factors consists of four dimensions, each with six items

Sub dimension	**Questions**
Social Skills	1,3,12,13,16,17
Social Support [a]	2,4,7,20,21,23
Goal Efficiency	6,9,10,11,14,19
Planning prioritizing behaviour , [a]	5,8,15,18,22,24

[a] Dimensions with corrective scoring for participants 84, 156, 235 and 238

For the Brief Resilience Scale, the sum of all responses was calculated per participant varying from 1-5 (Likert 5 scale) for all six items, providing a range of 6-30. The BRS has no sub dimensions. The total sum was then divided by the total number of questions answered to provide a mean score (Smith *et al.,* 2008).

For Adult Resilience Measure, the total and mean scores were calculated per each cluster, namely individual, personal relationship with key individuals and context/sense of belonging. Each cluster is further divided into sub scales, as seen in **Table 3.3**. To calculate the total scores, the sum of all responses were added within the cluster. The sum of each cluster was then divided by the total number of questions within the cluster to provide mean scores. The mean scores for each cluster range from 1-5. The total score ranges from 28-140 (Liebenberg *et al.,* 2012).

Table 3. 3 The Adult Resilience Measure consists of three clusters, further separated by sub-scales to measure aspects of resilience

Cluster	Sub scale	Questions
Individual	Personal skills	2,8,11,13,21
	Peer support	14,18
	Social skills	4,15,20,25
Personal relationship with key individuals	Physical caregiving	5,7
	Physiological caregiving	6,12,17,24,26
	Spiritual	9,22,23
Context/sense of belonging	Educational	3,16
	Cultural	1,10,19,27,28

Social Anxiety Questionnaire responses were calculated by adding all responses grouped within a dimension (total of 5 dimensions), shown in **Table 3.1**, with 6 items for each randomly distributed throughout the scale to provide a total score. The scoring ranges from 30-150 (Caballo *et al.,* 2012

Table 3. 4 Social Anxiety Questionnaire dimensions and respective items

Dimension	Social situation	Questions
1	Speaking in public/talking with people in authority	3,7,12,18,25,29
2	Interactions with the opposite sex	4,6,20,23,27,30
3	Assertive expression of annoyance, disgust or displeasure	2,5,9,11,14,26
4	Criticism/embarrassment	1,8,16,21,24,28
5	Interactions with strangers	10,13,15,17,19,22

State Trait Anxiety Inventory for Adults consisted of a Likert 4 scale, which used a reverse scoring method for specific questions (i.e response of 1 would score 4, response of 2 would score 3, response of 3 would score 2, response of 4 would score 1). The following questions had reverse scoring applied; 1, 2, 5, 8, 10, 11, 15, 16, 19, 20, 21, 23, 26, 27, 30, 33, 34, 36 and 39. Once reverse scoring was applied, responses were divided into those belonging to state and those that belonged to trait. The total sum of each was then calculated. Individual scoring ranges from 40-160 (Spielberger *et al.*, 1983, 1989; Shewchuk *et al*., 1998).

For the Beck Anxiety Inventory the total score for each participant was calculated by adding the sum of the 21 responses, using a 3 point Likert scale, with a score range of 0-63. These scores where then formatted to indicate those that fell into minimal (scores of 8-15), mild (scores of 16-25) and concerning high levels of anxiety (scores of 26-63). (Beck *et al.,* 1988).

To compute Childhood Trauma Questionnaire scores, the sum of the responses was added per abuse category (total of 5). Reverse coding was applied to the following questions; 5, 7, 13, 19, 28 (emotional neglect); 2 and 26 (physical neglect). Reverse coding used was as follows; response of 1 scored 5, response of 2 scored 4, response of 3 remained 3, response of 4 scored 2 and response of 5 scored 1. Each abuse category has a possible range of 5-25. A minimization/denial validity was included, where by 1 point was given to responses of 5 (very often true) for questions 10, 16 and 22, which are statements describing 'perfect childhood'. Question 10 states "There was nothing I wanted to change about my family", question 16 states "I had the perfect childhood" and question 22 states "I had the best family in the world". In relation to all other questions in the scale, these specific ones manage a denial factor based on the idea that no childhood is perfect in reality. The sum of the denial

score was added, with a possible range of 1-3, which is then subtracted from the respective participant scores (Bernstein and Fink, 1998; MacDonald *et al*., 2016). The sum of the scoring for each of the five sub dimensions ranges from 5-25, whereas the overall ranges from 25-125, with the addition of the denial score. Cut off scores, **Table 3.5**, were applied to determine abuse level percentages in the full sample group, ranging from an abuse level of none to severe. The levels for each category of abuse ranges, with emotional neglect starting at a higher range and sexual abuse starting at a lower range (Pennebake and Susman, 1988).

Table 3. 5 Childhood trauma questionnaire thresholding for abuse level categories

Abuse level	Emotional abuse	Physical abuse	Sexual abuse	Emotional neglect	Physical neglect
None	8	7	5	9	7
Low[a]	12	9	7	14	9
Moderate	15	12	12	17	12
Severe	16+	13+	13+	18+	13+

[a]Cut off score for abuse levels applied

Lastly for Adverse Childhood Experiences, every "Yes" response, was allocated a point of 1. The total was calculated by added the sum of each 1 point for 10 questions (Felitti *et al.,* 1998). A total of 4 out of the 54 participants has responses to the ACE questionnaire missing due to an error in the uploading of the Google form. This error was identified and corrected by removing the four participants from the ACE results. The missing responses could not be corrected, as done with the SPF scale due to the response method of "yes" or "no.

3.2.5 Analysis of data

Once scoring for all scales was complete these data were transferred to an Excel spreadsheet and transferred to Statistica version 13 (TIBICO Software Inc.) for statistical analysis. All data distributions were tested, using Shapiro-Wilks to indicate which data sets were grouped under parametric (normal distribution) and non-parametric (not of-normal distribution) respectively (**Appendix V**)

To determine sex differences, parametric data underwent independent t-tests, reporting t-test value and p-value, and non-parametric data underwent Mann-Whitney-U test, reporting z-test value and p-

value, when significant differences were found, p-value < 0.05. Due to scale scores being of mixed distributions, we report both mean with standard deviation and median with range for each of the scales.

Correlation analysis was applied between all scales to identify links between anxiety, resilience and childhood trauma. First, correlation analysis was performed between each total scale score. Once this was done, any scale which was divided into sub dimensions then underwent correlation analyses to identify if any of the functional behavioural categories between sub dimensions of the scales were related. These analyses was performed for the full sample group, and for each sex.

When both measures investigated were of normal distribution, Pearson's correlation co-efficient was applied, when one or both measures were of non-normal distribution, Spearman's rank order correlation was applied. All values where p>.05 were identified as significant for reported correlation between scales and/or sub dimensions (**Appendix V**)

3.3 Results

3.3.1 Population characterization

The sample population consisted of 17 males and 37 females, with average age of 21±1.66. Of the 54 participants whom completed the full online questionnaire, 28 identified as mixed race, 13 as black, 7 as white/European, 4 as South Asian (Indian), 2 as West-Asian, and 1 as South-East Asian. Three participants reported suffering from mental illness, of which two reported both anxiety and depression, and one reported anxiety only. All three participants indicated the use of medication prescribed by a medical doctor. None reported disabilities.

3.3.2 Resilience

No significant differences were reported between male and female within the resilience scales, **Table 3.6**. The individual mean scores captured for BRS report 24 participants within the low resiliency (1.00-2.99) category and 30 within the normal resiliency range (3.00-4.30), with none falling within the high resilience category (4.32-5.00) (Smith *et al.*, 2008).

Table 3. 6 Resilience scale and sub dimension scores

		Male (n=17)		*Female (n=37)*				
Scale	**Sub dimension**	**Mean**	**Std. Dev**	**Mean**	**Std. Dev**	**t-test**	**z-test**	***p-value***
Scale of Protective Factors 24	Social Skills	4.84	1.29	4.37	1.15	-1.36	-	0.18127
	Social Support	4.96	1.11	4.96	0.98	-0.31	-	0.76077
	Goal Efficiency	5.87	0.8	5.67	0.92	-	-1.53	0.12671
	Planning prioritizing behaviour	4.76	1.21	5.16	1.16	1.15	-	0.25458
	Total SPF	20.53	3.6	19.94	2.81	-0.65	-	0.51612
Brief Resilience Measure	Total BRM	2.95	0.55	2.95	0.49	0	-	1
Adult Resilience Measure	Individual	4.23	0.51	4.08	0.5	-1.08	-	0.2865
	Personal relationship with key individuals	4.19	0.71	4.21	0.68	-	-0.04	0.9702
	Context/sense of belonging	3.95	0.85	3.93	0.61	-	-0.53	0.59555
	Total ARM	115.41	17.29	113.57	13.72	-0.42	-	0.67462

Mean and std. dev for male (n=17) and female (n=37), with reported significant p value where p<0.05

Of the three resilience scales, ARM and SPF showed a strong relationship for the full sample group ($r_{(Pearson's(n=54)}$=0.54, p<0.00001), with males reporting a higher positive correlations ($r_{(Pearson's(Male(n=17))}$=0.69, p<0.00001; $r_{(Pearson's (Female (n=37))}$=0.45, p<0.00001), **Figure 3.1**.

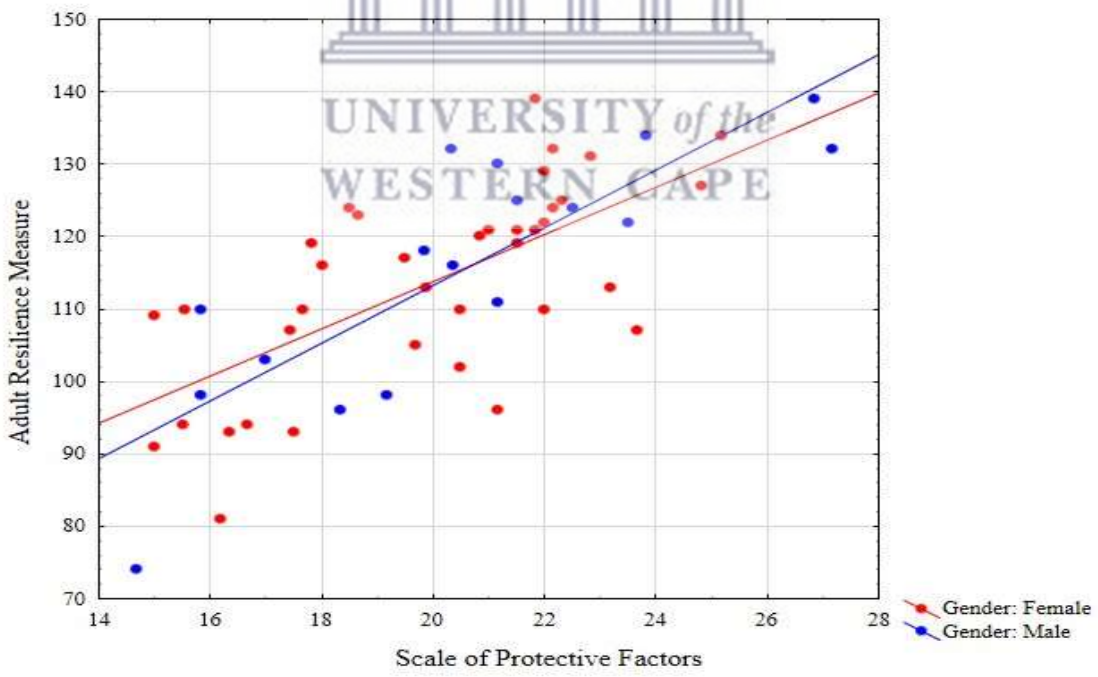

Figure 3. 1 Correlation between ARM and SPF on the full sample group. The Adult Resilience Measure and Scale of Protective factors scale showed a significant positive correlation with one another between sexes, with little differentiation between male and female.

3.3.3 Anxiety

Significant differences were reported between males and females for SAQ and BAI where females reported higher than males, while reported scores for State and Trait showed no difference between sexes, **Table 3.7**. SAQ was the only anxiety measure which included sub dimensions, where speaking in public/to authority and interactions with the opposite sex reported higher mean scores for females. For SAQ, speaking in public/to authority and interactions with the opposite sex reported the highest mean average, followed by interactions with strangers, suggesting these are two social dimensions which affect the sample group the most and cause social anxiety arousal.

Table 3. 7 Anxiety scale scores reported for male and females

		Male (n=17)		*Female (n=37)*				
Scale	**Sub dimension**	**Mean**	**Std. Dev**	**Mean**	**Std. Dev**	**t-value**	**z-value**	***p***
Social Anxiety Questionnaire for Adults 30	Speaking in public/to authority	18.82	3.81	22.51	5.58	2.47 [a]	-	0.01682
	Interactions with the opposite sex	18.65	4.3	24.03	4.72	- [a]	3.6	0.00036
	Assertive expression of annoyance/disgust/displeasure	17.88	5.58	19.78	5.82	1.13	-	0.26380
	Criticism/embarrassment	17.88	5.58	19.78	5.82	1.13	-	0.26380
	Interactions with strangers	18.29	5.07	20.65	5.86	1.43	-	0.15918
	Total	91.53	18.48	106.16	21.76	2.5 [a]	-	0.01568
Spielberger State Trait Inventory for Adults	State	18.06	4.8	23.76	7.43	2.89 [a]	-	0.00563
	Trait	17	4.57	22.22	5.77	3.28 [a]	-	0.00187
Beck Anxiety Inventory	Total	16.35	11.25	21.95	14.44	-	1.4	0.16246

Females reported higher mean scores for all scales

[a] test scores, where $p<0.05$

The average mean for BAI was 41.31± 13.98, indicating that the majority of the sample group are categorized as high levels of anxiety (Beck and Steer, 1993). A total of 31.48% of the sample group fell within the high level of anxiety bracket, followed by 27.78% moderate, 24.07% low and 16.67% minimal levels of anxiety. **Figure 3.2.** Females were found to have higher reported anxiety than males overall. Males accounted for 23.53% and females 13.53% in the minimal level of anxiety bracket. For severe levels of anxiety however, males accounted for 23.53% and females for 35.14%.

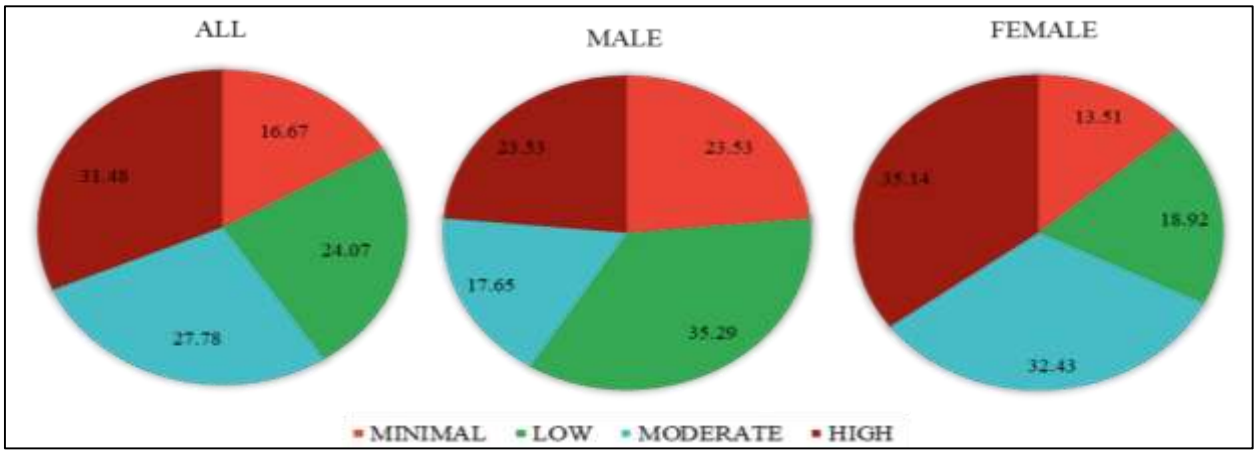

Figure 3. 2 Levels of anxiety for BAI for population and by sex. The levels of anxiety across sample group for the Beck Anxiety Inventory in the full sample group, male and female, where groups are categorized minimal (0-7), low (8-15), moderate (16-25), and high (26-63) anxiety.

All three anxiety scales showed strong correlation to one another, SAQ and STAI-State ($r_{(Pearson's(n=54)}$=0.17, p=0.0021); SAQ and Trait ($r_{(Pearson's(n=54)}$=0.24, p=0.0002); STAI-State and BAI($t^2_{(Spearman's(n=54)}$=0.53, p=0.00004); and STAI-Trait and BAI ($t^2_{(Spearman's(n=54)}$=0.53, p=0.00004), (**Figures 3.3-3.6**), all reporting strong positive correlations

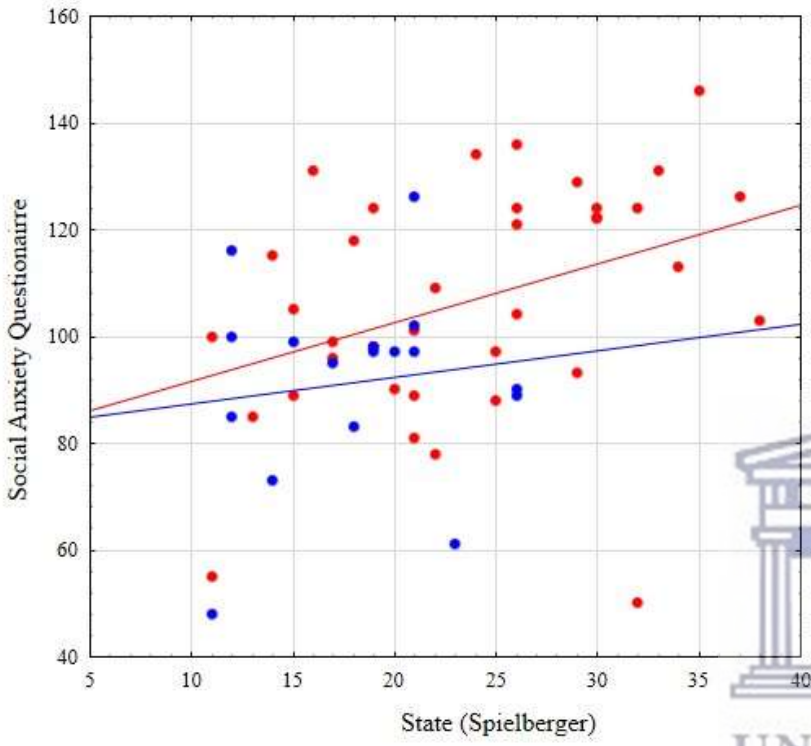

Figure 3. 4 Correlation between SAQ 30 and STAI-State on the full sample group. Significant correlation between the Social Anxiety Questionnaire and the State section of Spielberger's State Trait Anxiety Inventory showed a positive relationship for both sexes, with females showing a slightly stronger correlation between the two scales.

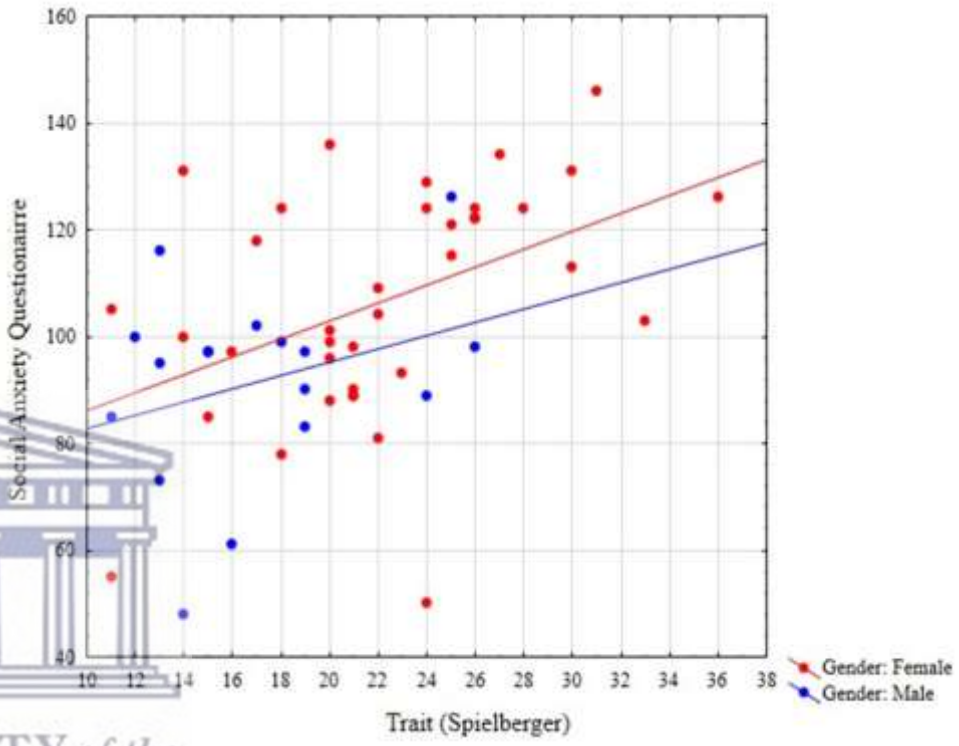

Figure 3. 3 Correlation between SAQ 30 and STAI-Trait on the full sample group. Significant correlation between the Social Anxiety Questionnaire and the Trait section of Spielberger's State Trait Anxiety Inventory for both sexes.

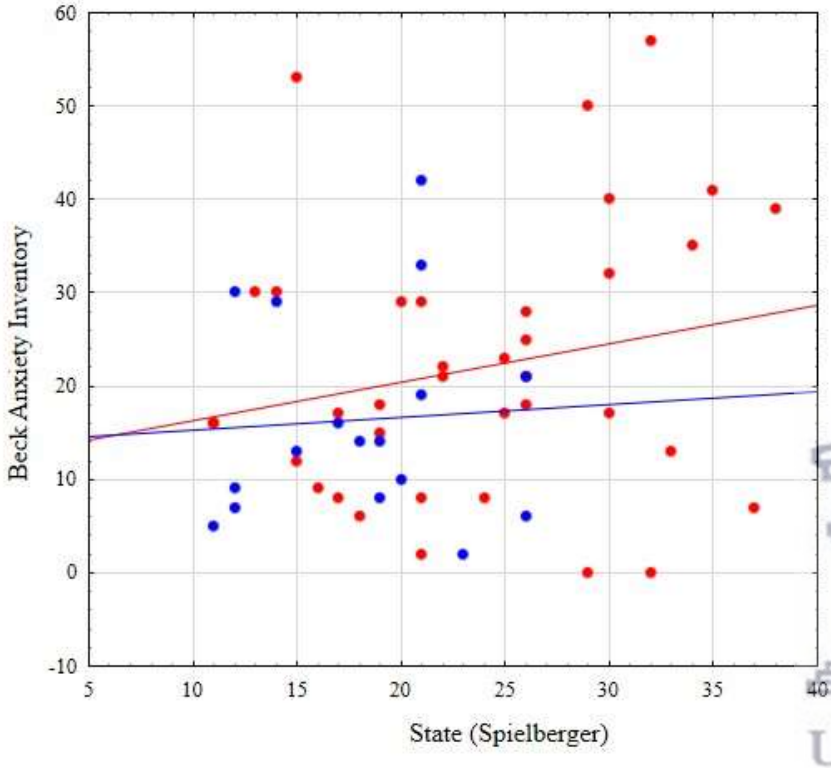

Figure 3. 6 Correlation between BAI and STAI-State on the full sample group. The correlation between the Beck Anxiety Inventory and the State section of Spielberger's State Trait Anxiety Inventory for both sexes. Males show a lack of correlation between the two scales.

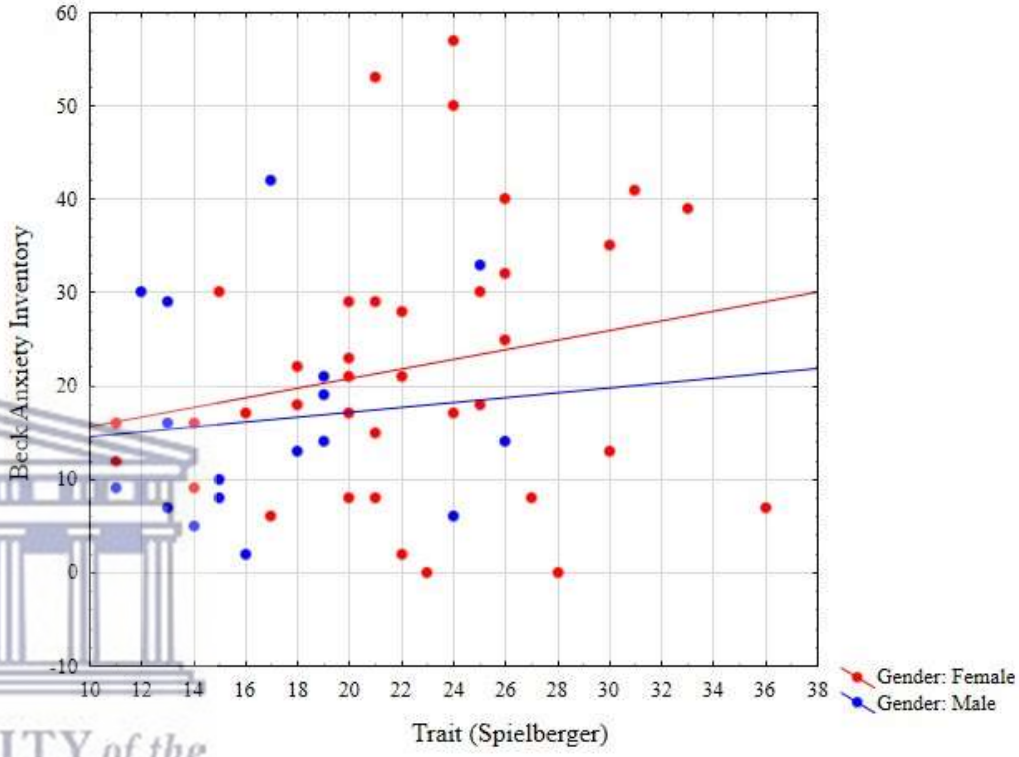

Figure 3. 5 Correlation between BAI and STAI-Trait on the full sample group. The correlation between the Beck Anxiety Inventory and the Trait section of Spielberger's State Trait Anxiety Inventory for both sexes. Females display a stronger relationship between the two scales

3.3.4 Childhood trauma

The only significant sex difference across adverse childhood scales, the CTQ and ACE, was reported for physical abuse subscale of the CTQ, where physical abuse was greater in males compared to females, **Table 3.8**.

Table 3. 8 Childhood trauma scale scores for both sexes

		Male (n=17)		*Female (n=37)*			
Scale	**Sub dimension**	**Mean**	**Std. Dev**	**Mean**	**Std. Dev**	**z-value**	***p***
Bernstein Childhood Trauma Questionnaire	Emotional abuse	8.06	3.45	9.3	4.59		
	Physical abuse [a]	7.47	2.45	6.16	1.59	-2.1	0.04330
	Sexual abuse	6.59	4.02	6.22	2.84		
	Emotional neglect	9.53	3.57	8.95	3.66		
	Physical neglect	7.71	2.34	6.97	2.24		
	Total	39.18	12.74	36.97	9.78		
		(n=16)		*(n=34)*			
Adverse Childhood Environment [a]	Total	1.94	2.41	2.59	2.61		

[a]: *N* value for ACE changed due to removal of incomplete responses, for male (*n=16*), female (*n=34*)

To better understand the percentage of the sample group which indicated abuse on the CTQ, a count was done on the sample group to categorize those that experienced 0-5 types of abuse. The minimum cut off value of "low" was applied across all dimensions. The cut off values were; 12 for emotional abuse, 9 for physical abuse, 7 for sexual abuse, 14 for emotional neglect and 9 for physical neglect (Bernstein and Fink, 1998).

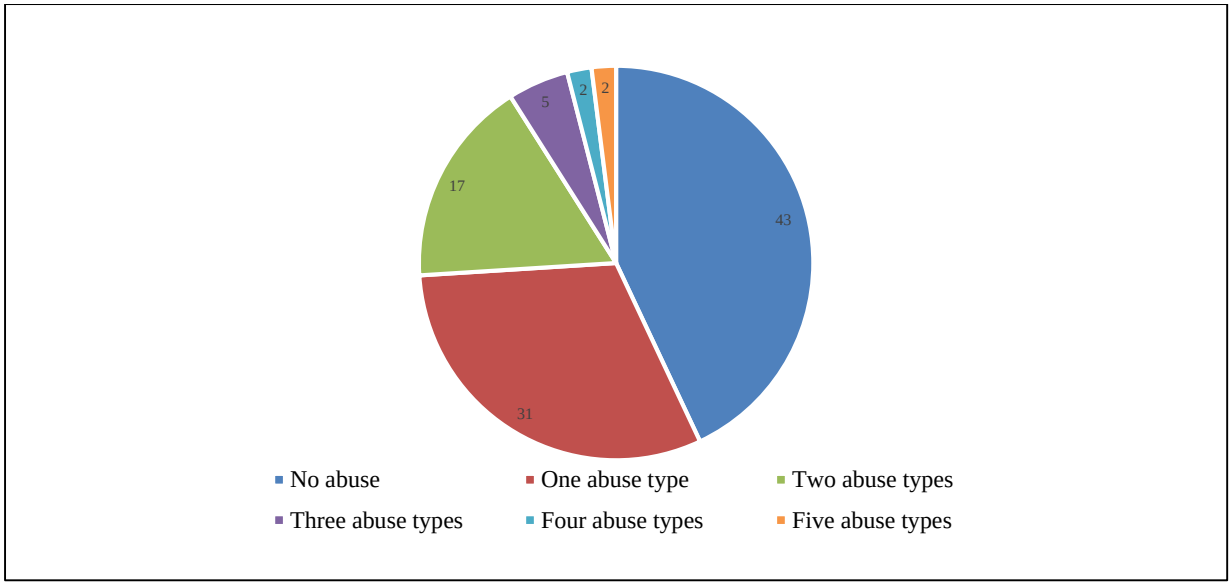

Figure 3. 7 The levels of abuse for CTQ ranged from no abuse to five abuse types. Participants experienced a number of abuse types as measured using the Childhood Trauma Questionnaire (CTQ). The highest percentage of the sample group (43%) reported nominal childhood abuse, while 31% reported at least one type of abuse, 17% reported two, 5% reported three, 2% reported 4, and 2% reported all the forms of abuse captured by the CTQ.

A positive correlation was reported between CTQ and ACE ($r_{Spearman's\ (n=50)}$= 0.55, p=0.000034) (**Figure 3.8**), with females showing a significantly stronger correlation between CTQ and ACE than males.

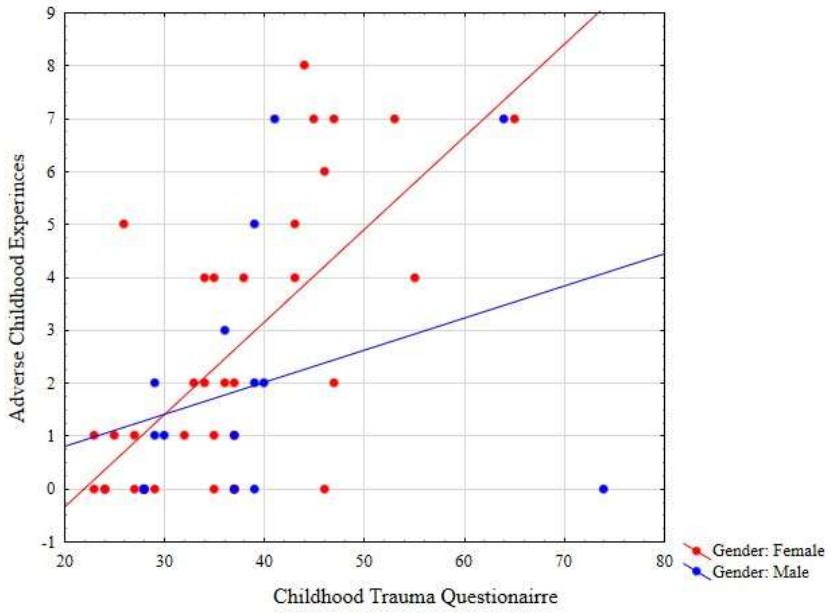

Figure 3. 8 Correlation between CTQ and ACE for the full sample group. The correlation between the Childhood Trauma Questionnaire and Adverse Childhood Experiences for both sexes. Females are noted to have a significantly stronger correlation.

3.3.5 Correlation of scales across functional behavioural categories

For the full sample group, STAI-trait related to all total scale scores except ACE ,**Figure 3.9**, where it correlated to STAI-state as expected as it falls within the STAI ($r_{Pearson's(n=54)}$=0.69, p<0.00001); CTQ ($r_{Spearman's(n=54)}$ = 0.34, p=0.01); ARM ($r_{Pearson's(n=54)}$=0.10, p=0.02); BRS ($r_{Pearson's(n=54)}$=0.10, p=0.02) and SPF($r_{Pearson's(n=54)}$=0.14, p=0.01),

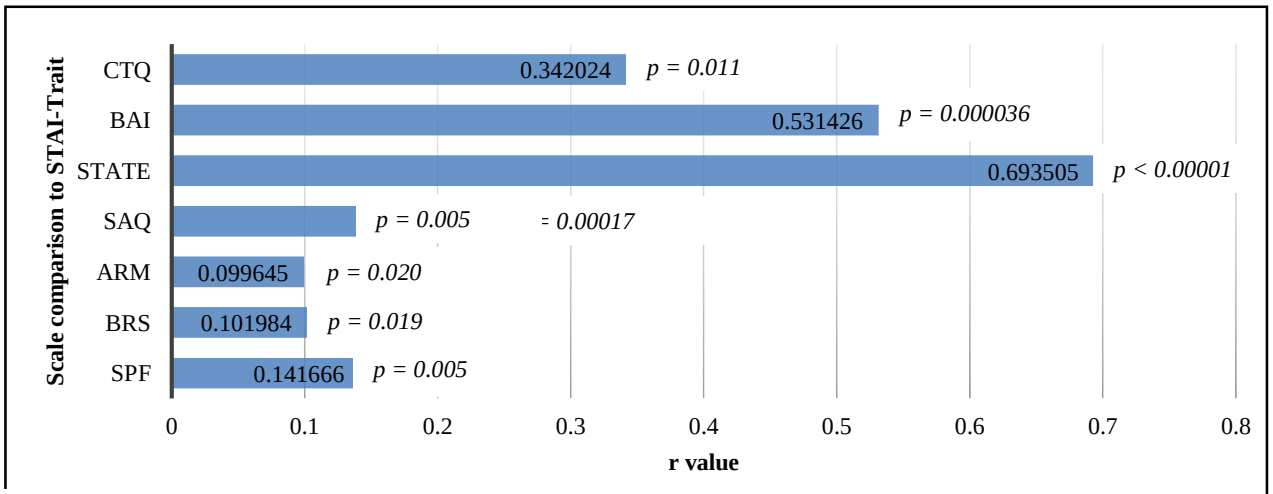

Figure 3. 9 The STAI-Trait correlated to all scales besides ACE. The Trait section of the Spielberger's State Trait Anxiety Inventory reported good correlation with all other scales in the full sample group, excluding the Adverse Childhood Experiences.

Between the functional behavioural categories of anxiety, resilience and childhood trauma; BRS and STAI-State reported a positive correlation ($r_{Pearson's(n=54)}$= 0.080, p=0.037) ,**Figure 3.10**. ARM and CTQ reported a negative correlation ($r_{Spearman's(n=54)}$=-0.45, p=-0.00065), **Figure 3.11**.

Reporting correlation within the sub dimensions of the scales, two sub dimensions of scales showed significant correlation where the sub dimension "individual" of ARM and "goal efficiency" of SPF ($r_{Spearman's(n=54)}$)=0.50, p<0.00001); and "personal relationship with key individuals" of ARM and "emotional neglect" of CTQ ($r_{Spearman's(n=54)}$=-0.65, p<0.00001), **Figure 3.12**. In addition, two other correlations were observed which are important to note; "goal efficiency" of SPF and "context/sense of belonging" ($r_{Spearman's(n=54)}$=-0.47, p=0.0003); and "planning and prioritizing behaviour" of SPF and "context/sense of belonging" of ARM ($r_{Spearman's(n=54)}$=-0.48, p=0.0002).

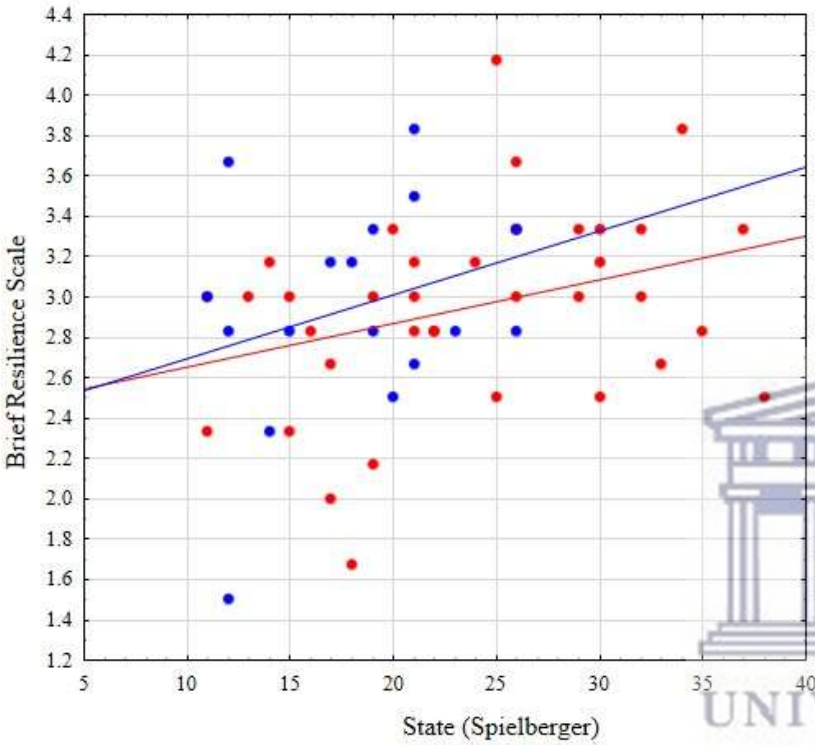

Figure 3. 11 Correlation between STAI-State and BRS on the full sample group. A positive correlation between the State sections of Spielberger's State Trait Anxiety Inventory and the Brief Resilience Scale for both sexes.

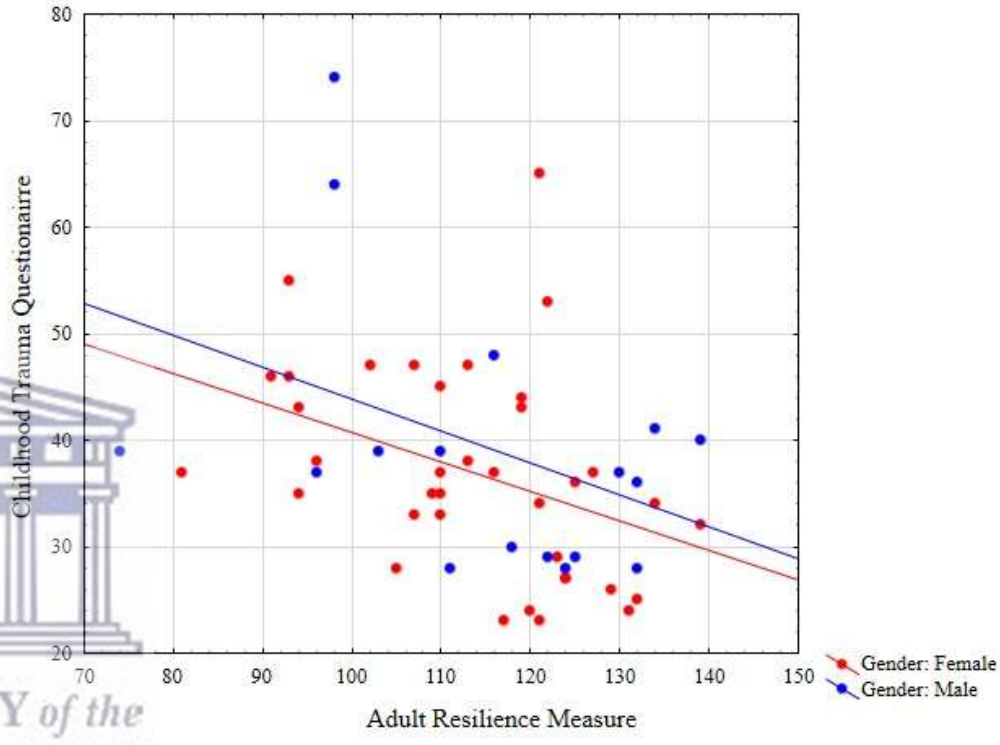

Figure 3. 10 Correlation between ARM and CTQ on the full sample group. A negative correlation between the Adult Resilience Measure and the Childhood Trauma Questionnaire for both sexes.

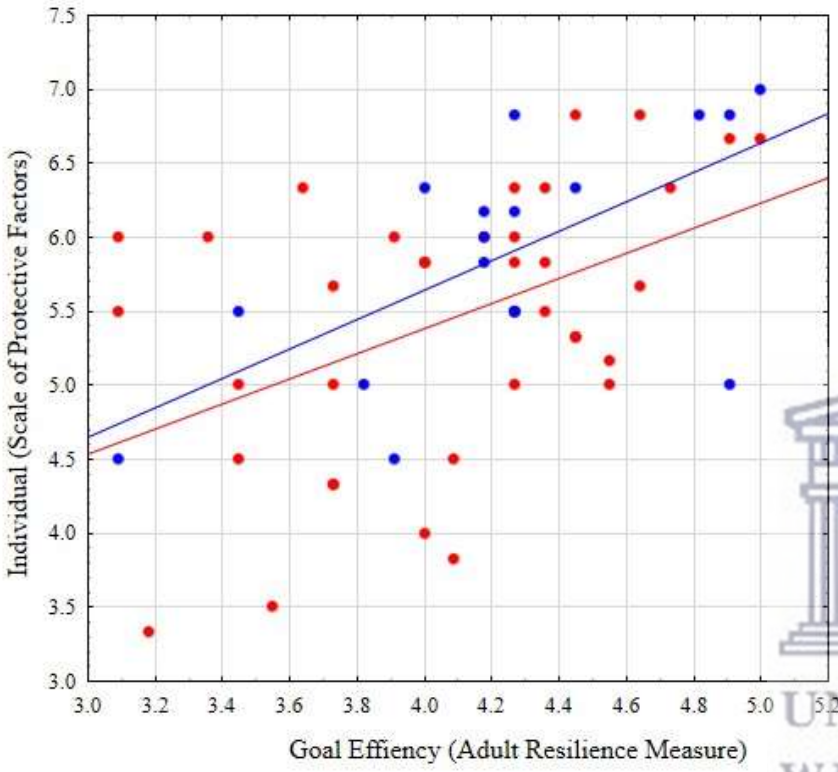

Figure 3. 13 Correlation between the sub dimension of "individual" of the Adult Resilience Measure and "goal efficiency" of Scale of Protective Factors. A positive correlation for both sexes was reported.

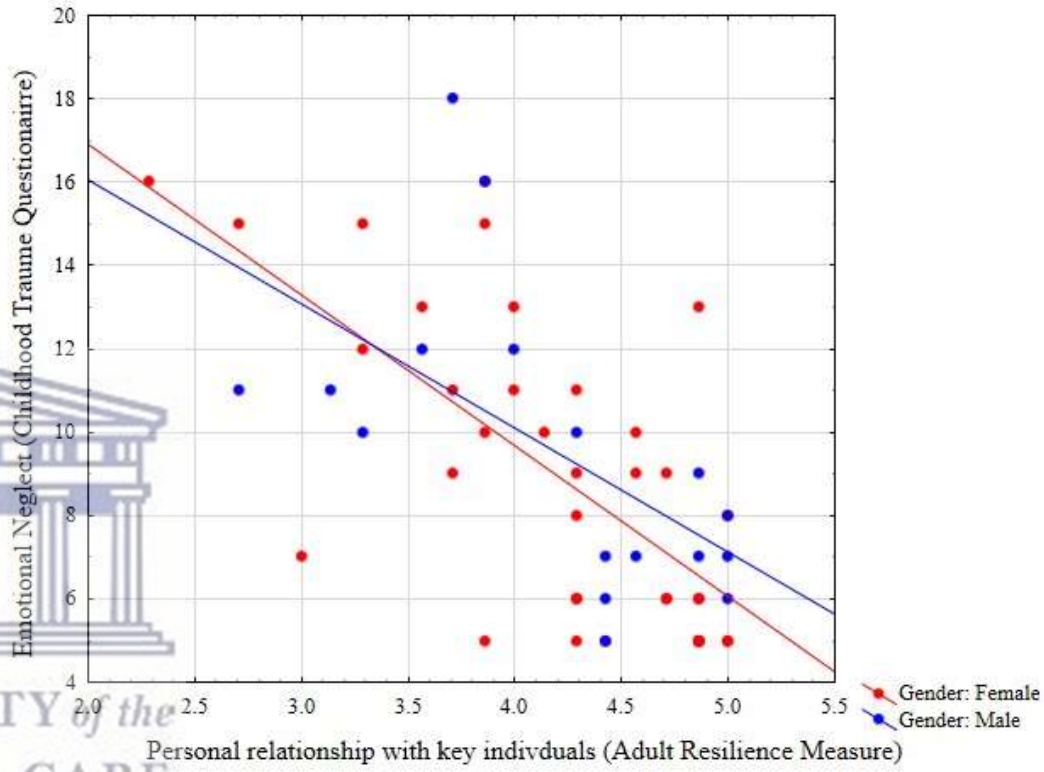

Figure 3. 12 Correlation between the sub dimension of "personal relationship with key individuals" of Adult Resilience Measure and "emotional neglect" of the Childhood Trauma Questionnaire. A negative correlation for both sexes was reported.

3.4 Discussion

The investigation into the relationship between three functional behavioural categories, namely resilience, anxiety and childhood trauma; and the role of sex, was achieved with the use of self-report measures and correlation statistics. The results for this study provide insight in several ways, namely; that resilience is independent of sex, yet anxiety shows variability between sexes with females linked to higher reported levels. All three functional categories: resilience, anxiety and childhood trauma are associated which suggests possible interplay between childhood trauma, anxiety, and resilience, however would require further validation through more in depth analysis.

3.4.1 Resilience is independent from sex

Differentiation between sexes in measures of resilience, namely SPF, ARM and BRS scales, were not apparent for the sample group which align with current literature. In a study by Laor *et al*., (2006) similar levels of resilience between males and females were reported, which was later supported by the findings of Dubow and colleagues in 2010 (Dubow *et al*., 2010; Laor *et al.,* 2006). A recent study which assessed resilience amongst 2604 first year undergraduate university students in Malaysia reported no statistical difference in resilience between male and female (Ahmad *et al*., 2018).

However, some studies have observed that females score lower on measures of resilience, with higher rates of exposure to stressful events, while more than often making use of emotion-based coping methods; in comparison to men (Stratta *et al*., 2013; Greenglass, 2001). As suggested by Sun and Stewart (2007), it is important to consider that gender differences in resilience is influenced by not just age, but also the difference in personalities between men and women which manage the way in which one copes with adverse environments (Sun and Stewart, 2007). Despite the many studies which look at resilience between male and female, gender has been deemed both an inconsistent and unreliable resilience marker (Ballenger *et al*., 2010).

Higher resilience levels in females have been reported by some, mostly observed amongst older women, suggesting that although there may be no difference in resilience amongst male and female, age could play a considerably important role (Netuveil *et al*., 2008).

Differences between male and female, which develop during childhood into early adolescent life influence the way in which individuals attain characteristic of resilience. These differences include coping mechanisms, which differ between sexes in stressful situations (Cohen *et al.*, 2003). In the face of adversity, males are more likely to rely on their independence, in contrast to females who make use of support systems instead, thus when exposed to adverse conditions the coping mechanisms differ between male and female and therefore their path toward resilience differs too (Sneed *et al*.., 2006). With resilience being such a complex and multidimensional construct, it is important for researchers to consider all variables and factors which embody resilience as a functional behavioural category which include age, gender, epigenetics and exposure to childhood trauma.

3.4.2 Anxiety varies between male and female

Variation in anxiety levels between sexes appear to be indicative of the anxiety classification, specifically in social anxiety dimensions where females reported higher levels. Females appear to have higher levels of GAD, when taking into consideration the results produced from the BAI. However the significance of this difference between sexes warrants for further investigation.

Differences between sexes in anxiety has been inconsistent throughout research, with some studies showing differences between male and female, while others report no significant differences. In the SAQ scale, no difference was seen within both sexes for both assertive expression of annoyance/disgust/displeasure and criticism/embarrassment, reporting equally in both dimensions. This indicates that interactions with the opposite sex has the greatest impact on the sample group in causing anxiety arousal, following closely by speaking in public/individuals in authority. Interactions with strangers; assertive expression of annoyance/disgust/displeasure and criticism and embarrassment showed an even spread but lower anxiety arousal, suggesting these are areas of social engagement that do not affect the sample group greatly in comparison to speaking to the opposite sex or authority

Lee and Kwok (2005) confirmed the findings by Bourdon and co-authors (1998), which reported no significant differences in anxiety, specifically SAD, between sexes. However, there are many studies which reported SAD to be a more common occurrence in women than men (Kessler and

Stein, 1998). In 2014 Cabello and colleagues carried out a population based study on self-report scales for anxiety in men and women, reporting women to have significantly higher scores then men; confirming previous studies by several other authors (Cabello *et al.*, 2014; Hirai *et al*., 2011; Baños *et al*., 2007). Not all studies which reported women scoring higher than men were of significance, in fact some studies have reported the level of scoring in difference between sex to be of a non-significant level (Stewart and Mandrusiak, 2007; Hirai *et al*., 2011).

The differences reported between sex in both state and trait, as well as SAQ coincide with previous studies of women reporting higher trait and social anxiety (De Vore and Pritchard, 2013). In 2011, McLean and colleagues reported women to have higher rates of chronic anxiety, however found that social anxiety showed no difference between males and females in prevalence; and further reported that women who were diagnosed with lifetime anxiety were also more likely to be diagnosed with a second disorder such as depression or bulimia (McLean *et al.,* 2011).

Although McLean *et al*., (2011) reported no difference in social anxiety between sex, Asher and Aderka (2018) concluded after their 12 month study, that women are more likely to suffer from SAD (63.3%). They found that where women were more likely to suffer from GAD, PTSD and comorbidities; men were more likely to suffer from conduct and comorbid substance abuse disorders (Asher and Aderka, 2018). GAD is suggested to be more prevalent amongst females, individuals of low socioeconomic status, individuals who have had adverse childhood experiences, and individuals who have a family history of anxiety related disorder (Moreno-Peral *et al.*, 2014).

The SAQ, consists of several dimensions, with the two of the dimensions reported to have differences between sex; namely interactions with the opposite sex and speaking in public/to authority. In a recent study by Ejaz *et al*., (2020) which investigated the scale and scope of social anxiety in university students in Pakistan, they looked at sex differences in all dimensions of social anxiety using numerous anxiety self-report scales. They found that in certain social dimensions females scored much higher, namely in the participation of social events and tasks. One particular point highlighted by the authors was that women had reported fear in numerous social situations, which surfaced in 1999 in a study by Wittchen and co-authors. These situations included

communicating with the opposite sex, embarrassment and being observed by others (Ejaz *et al.*, 2020, Wittchen *et al.*, 1999).

The majority of the sample group fell within the bracket of concerning high levels of anxiety reported under the BAI, seen mostly amongst females, with females accounting for 35.14% of severe levels of anxiety. In a study by McLean *et al.*, (2011) consisting of more than 20,000 US citizens, females reported higher rates of all anxiety disorders on a lifelong scale. Later, in a 2014 cohort study by Wesselhoeft and colleagues, it was discovered that prior to puberty boys reported higher anxiety rates (Wesselhoeft *et al*., 2014). A 2017 review study by Hantsoo and Epperson on anxiety at different time points in the female lifespan suggested that the distinctive periods of hormonal function correlate to different representations of anxiety and provides context for the possible foundation of anxiety in females. In addition is it known that anxiety fluctuates in women, aside life span related: puberty and menopause, there are fluctuations related to pregnancy and child-rearing, and menopause (Hantsoo and Epperson, 2017).

In 1994 Nolen-Hoeksema reported higher levels of anxiety in females. More recent studies have also reported higher levels of both anxiety and depression in females in early adolescence, suggesting that difference between in anxiety emerges in the early stages of adulthood (Cullerton-Sen *et al*., 2008; Nolen-Hoeksema, 1994).

3.4.3 Childhood trauma and anxiety

The reported results for physical abuse in the CTQ scale was lower than expected. This dimension was expected to have a higher than average result due to the high rate of abuse in South Africa. South Africans are reported to have higher rates of childhood abuse and trauma irrespective of sex, which have been associated to the high rates of substance abuse (Stansfeld *et al.,* 2017; Hogarth *et al.,* 2018).

A study which investigated the factor structure of CTQ in 231 young adolescents in Burundi, Africa reported an abuse and neglect rate ranging from 14.7-93.5%, with more than 37% reporting four or more types of abuse and neglect. Emotional abuse and neglect, as well as physical neglect were reported to be 2-3 times higher in the sample group in comparison to other studies which

made use of the CTQ in higher income countries. In addition, those that reported four or more types of abuse also reported more symptoms of depression and PTSD (Charak *et al.*, 2017).

Researchers have found that males and females experience trauma at different rates, suggesting that there is a difference in both the rate and response to different traumas between sex (Tolin and Foa, 2006). Cullerton-Sen *et al.*, (2008) advised the importance of studies on emotional maltreatment and trauma with sex differences, with numerous studies having reported differences

A 2018 population based birth cohort study, consisting of 3778 mother and children pairs reported high association between childhood maltreatment and early adult mental health, with anxiety (PTSD) showing the strongest association. Emotional abuse and neglect were reported to have the most adverse effects on mental health, and children which experienced more than one trauma type were at higher risk (Kisely *et al.*, 2018). A total of 17% of the sample group having reported more than one type of abuse, which is a considerably high number, suggesting that this portion of the sample group is at higher risk of developing mental health conditions.

A study by Garicia and colleagues (2016) reported no sex differences in CTQ scoring, yet reported significant correlations in females with respect to CTQ scores with positive/negative psychotic and depressive symptoms; and weak functionality. Garica further reported associations of adverse childhood experiences and poor social cognition in both sex (Garica *et al*., 2016). Over recent years, childhood trauma studies investigating underlying illness and sex difference report significant differences between sex, which varies with the respective dimension and focus of the study.

The functional categories of anxiety and childhood trauma did not show correlation, which contradicts the abundance of research which link the two, with childhood trauma long considered to be a risk factor for the development of anxiety in adulthood. Emotional abuse and physical and emotional neglect have been associated to anxiety disorders (Kascakova *et al.*, 2019).However, Charney (2003) points out that the number of children whom do not develop an anxiety disorder later in life, despite childhood trauma suggests other biological and genetic factors (Charney,

2003). These factors could include the development of resiliency and genes such as *COMT*, which show both a direct and indirect involvement in anxiety.

There was however a correlation between childhood trauma and resilience, suggesting that perhaps resiliency has served as a protective factor from anxiety in the sample group. Children that have been exposed to adverse childhood experiences or PTSD are known to be more susceptible to anxiety, with studies suggesting prior avoidant personality characteristic to be a risk factor (Biederman *et al.*, 2001 Rapee, 2002).

3.4.4 The correlation between resilience and childhood trauma

Resilience in context of trauma is defined in several ways based on an ecological approach, which include positive outcomes despite exposure to adverse childhood experiences, the prevention of reoccurrence of trauma despite the possible risk of further trauma exposure and the avoidance of trauma experiences in the face of significant risk (Bartlett and Steber, 2019). This ecological approach is defined by the promotion of resilience in individuals who experience early trauma, with the understanding that there are multiple levels which influence resilience, namely risk and protective factors, which have been incorporated in most resilience and childhood trauma measures as sub scales (Bartlett and Steber, 2019).

The measures of childhood adversity and resilience, specifically CTQ and ARM, showed a strong positive correlation, supporting the association between the two as functional behavioural categories. There are a number of factors which contribute to resilience in adulthood following adverse childhood experiences and trauma. These factors include support systems from friends and family, the feeling of safety both inside and outside the home, access to resources which assist in safeguarding one from negative situations, possessing an overall positive self-worth and having a sense of meaning in life which includes connections with others, aspirations and goals. Exposure to adversities such as life in poverty, community violence and social isolation can undermine resilience (The National Child Traumatic Stress Network, 2019).

The above mentioned factors commonly lack within the childhood of South Africans. A 2015 publication on the lives of South Africa children reported that of the 18.5 million children under the age of 23% do not live with their biological parents, 60% live in poverty and 8% do not have

a father. It was further reported that 5% of children admitted to Red Cross Children's Hospital in Cape Town annually is due to physical or sexual abuse cases (Bateman, 2015).

The high reported number for child and female abuse, along with the lack of sufficient support systems and resources in communities lead to a decrease in self-worth, aspirations and goals in many individuals. A 2019 study by Manyema and Richter on adverse childhood experiences in young South African adults showed adverse experiences to be highly prevalent in middle income households (Mayema and Richter, 2019). However, poverty and community violence is high in lower income households, with 40% of the population living below the upper bound poverty line (Statistics South Africa, 2020).

In a longitudinal study on 700 individuals in Kauai, with 33% reporting adverse childhood conditions such as poverty or parental divorce, a total of 31% of the children were reported to be resilient as they developed into well-adjusted adults. It was further reported that there are a number of protective factors which lead to the resiliency of children exposed to adverse childhood conditions, which includes having a supportive system (Werner, 1997).

The sub dimension of "personal relationship with key individuals" of the Adult Resilience Measure and "emotional neglect" of the Childhood Trauma Questionnaire were positively associated. This aligns with research which shows the association between emotional neglect and interpersonal difficulties during childhood and adolescent life (Walker and Holman, 2009). Further elaborating on the association between emotional neglect and interpersonal difficulties, Walkman and Holman (2009) reported that an individual's perception of a past experience negatively impacts the present, thus is considered to be an important intermediary between negative childhood experiences and the quality of relationships in adolescents (Walker and Holman, 2009).This reported association between emotional neglect and interpersonal difficulties holds value into the understanding of how negative experiences during childhood can impact the way in which individuals respond and react to relationships of those around them, with a sense of suggested resiliency.

Observing the positive correlation between childhood trauma and resiliency, suggests that resiliency may serve as a form of protective factor from anxiety related disorders. A 2016 study

which investigated self-resilience as a protective factor amongst 112 male police officers who had experienced a traumatic event found that officers with low-self resilience had higher rates of PTSD than those with high self-resilience, concluding that a high degree of self-resilience may protect officers from incident related PSTD symptoms (Lee *et al.,* 2016).

In 2014 Vandevender reported that individuals who were exposed to childhood maltreatment, such as emotional neglect, not only struggle to start relationships but also to establish form of independence within these relationships. It was further reported that sex may in fact serve as a moderator for the relationship between adverse childhood experiences and interpersonal outcomes, as males which reported maltreatment during childhood had lower levels of emotional support (Vandender, 2014).The correlation found between the sub scales of resilience and childhood trauma, namely ARM and CTQ, align with previous literature which suggest the linkage between the two, as complex multidimensional construct.

3.5 Conclusion

All three functional behavioural categories were significantly related, with a strong associations between childhood trauma and anxiety, as well as childhood trauma and resilience. Anxiety appears to differ between sexes, with females experiencing higher levels than males, particularly in general and social anxiety situations. It is important to take into consideration the time points in the female lifespan varies with regards to hormonal function, which could serve as a reasoning as to why females are often found to experience and report higher levels of anxiety related symptoms (Hantsoo and Epperson, 2017).

Although there was no clear association between childhood trauma and anxiety, previous literature links early exposure to trauma and adversities to psychological disorders ranging from depression to anxiety. The poverty and unemployment rate in South Africa has led to harsh living conditions for most of the population, with many middle to lower income communities experiencing traumatic events which include substance abuse, violence, crime, physical abuse and a lack of social support. Many children experience more than one type of trauma early in life, which increases the risk of developing a mental health condition.

However, not all children who are exposed to traumatic events during childhood develop a psychological disorder later in life, which suggests that there are other contributing factors which could buffer or halt the development of such disorders. Both genetics and resilience have been thought to be possible contributing factors. A strong correlation between childhood trauma and resilience was observed in the sample group, suggesting that perhaps resiliency has served as a protective factor from anxiety.

Goal efficiency (ARM) and personal relationships with key individuals (CTQ) showed correlation for resilience and childhood trauma, suggesting an association between the two as functional behavioural categories. Differentiation between sexes in measures of resilience were lacking. Gender differences in resilience is influenced by not just age, but also the difference in personalities between men and women which manage the way in which one copes with adversities. In this study our data does not support a separation in the development of resilience for either sex. It is integral, with resilience being such a complex and multidimensional construct, that researchers continue to consider potential factors which may serve to influence the development of resiliency.

Limitations the study include the low sample number and uneven distribution between male and female, A higher sample number would be of a higher statistical value, providing stronger based evidence for the findings. Equal distribution of sex for the number of participants would have provided a more fair and unbiased overview of the measures. Due to the study making use of only self-report scales, none of the samples were classified within the anxiety spectrum clinically by a medical health professional. The use of the self-report scales namely; were used as a means of correlating scoring to the pilot genetic analysis for the purpose of better understanding and insight into the association of *COMT* SNPs with the functional behavioural categories in a general student South African population, to be discussed in the next chapter (4).

A clear relationship exists between all three function categories namely; anxiety, resilience and childhood trauma as they reported correlations in various dimensions independent of one another. STAI- State, a reputable and well used scale for measurement of anxiety, correlated to all other scales, except ACE, showing that there is an association between the functional categories. Studies link both anxiety and resilience to childhood trauma, with childhood trauma acting as an indirect

possible buffer to the development of anxiety disorders due to resiliency in some individuals. To understand what determines this resiliency to develop in only some who have had adverse childhood experiences needs to be further investigated, with the possibility of taking genetic factors into consideration.

CHAPTER 4

The relationship between *COMT* SNPs and self-report measures of anxiety, resilience and childhood trauma

4.1 Introduction

There is an abundance of research on singular *COMT* SNPs and their association to anxiety, resilience and childhood trauma, of which rs4680 (Val ^{158} Met) appears to be the most common. Baumann and colleagues (2013) investigated how the interaction with early life experiences and rs4680 affects anxiety sensitivity. The results reported support the idea of a gene-environment interaction between *COMT* and childhood adversity in relation to anxiety. They found that exposure to adverse childhood experiences lead to an increased risk for anxiety in individuals which are homozygous for the rs4680 *COMT* Met allele (Baumann *et al.*, 2013).

Despite an abundance of studies reporting association between *COMT* variants and anxiety related disorders, resilience and childhood trauma, the mechanisms which underline this gene-disorder association are not fully understood. It has been suggested that following exposure to trauma during childhood, the polymorphisms of *COMT* affect brain structure and function or related cognitive processes, which lead to an increased risk for development of psychopathology or enhanced resilience (van Rooij *et al*., 2016). Verbuggen and Logan (2008) describe cognitive processes affected by gene polymorphisms, such as that of *COMT* and childhood trauma, as response inhibition; which is defined as the ability for an individual to supress a behaviour no longer required or inappropriate in a given environment (Verbuggen and Logan, 2008). A number of studies which have reported response inhibition to be linked to a range of psychiatric disorders, have suggested response inhibition to be mediated by the PFC (Sjoerds *et al*., 2014; Zandbelt *et al*., 2011).

In this chapter we draw together the empirical data, while reviewing the literature and exploring the data where both *COMT* SNP and self-report data were collected for measures of anxiety, resilience, and childhood trauma for male and female. Currently, no studies have investigated the *COMT* SNPs selected in this study as a possible haplotype for anxiety, resilience and childhood trauma; or the differentiation between sexes in a South African sample group.

4.2 Comparative analysis of *COMT* SNPs with self-report scoring for anxiety, resilience and childhood trauma

In this study we obtained *COMT* SNP data were obtained from 11 participants, **Table 4.1**, of which four completed the series of self-report measures. Three of the participants were found to have C-A-C-G-A-A, these three participants were also male. The fourth participant was found to have C-G-C-G-A-A, where rs4680 differed and displayed a G in place of A. The profile of rs4680 in the eleven participants showed an equal distribution of G and A without sex distinction, while all other *COMT* SNPs profiles were similar. This led us to explore the *COMT* SNP C-A-C-G-A-A, 3 participants, to the remaining participants (*n=50*) where *COMT* SNP profiles were not obtained but subjective measures were collected.

Table 4. 1 Identified alleles for COMT SNP's in the 11 samples genotyped

Sample ID	Sex	rs4633	rs4680	rs4818	rs6269	rs737865	rs2075507
10	M	-	G	G	A	A	A
22	F	-	G	C	G	A	A
40	M	-	G	C	G	A	A
60	M	-	G	G	A	A	A
76	M	C	A	C	G	G	A
78	F	C	A	C	G	A	A
80	M	C	A	-	G	A	A
83 [a b]	M	C	A	C	G	A	-
85 [a]	M	C	A	C	G	A	A
90 [a]	F	C	G	C	G	A	A
92 [a]	M	C	A	C	G	A	A

[a] participants which completed the self-report questionnaire
[b] *No observed peak was peak was identified for SNP rs2075507 in sample 83, p>0.05*

4.2.1 The association between COMT and anxiety

Heritability approximation ranges from 30-40% for GAD, with the suggestion that genetic and epigenetic factors play a key role (Hettema *et al*., 2005). A number of genetic studies have used candidate gene (hypothesis-based) and/or genome wide association studies (GWAS) (hypothesis-free) methods in an attempt to identify the genes that may be associated to anxiety disorders, which has not been very successful (Tomasi *et al.,* 2019). A 2008 study investigated *COMT* genes with

susceptibility to developing anxiety related phenotypes such as neuroticism, general anxiety disorder, and major depression; tested several *COMT* SNPs in 589 cases and 539 controls. The authors concluded that haplotype analysis for *COMT* increased susceptibility to anxiety related phenotypes, more so in females, suggesting the importance of sex-specific analysis. The authors observed the G-A haplotype, formed from the G allele of rs4680 and A allele of rs165599, which showed a significant association in the entire female sample, proving to be a better predictor than rs4680 for anxiety and neuroticism (Hettema *et al.,* 2008). In an earlier study which also looked at the association between *COMT* and anxiety in a mixed ethnic sample of 497 university students, a strong association was found between the haplotype G-A-A for rs737865-rs4680-rs165599 and dichotomous neuroticism in females (Stein *et al.,* 2005).

Due to anxiety being more prevalent amongst females, reports of a possible haplotype linked specifically to females is an area to be further explored. Hettema and colleagues hypothesized that although not all studies have been in agreement of the differentiation between sex, the difference in estrogenic hormones between male and female could influence the suggested haplotypes (Hettema *et al*., 2005).*COMT* has an estrogenic response in the promoter region of the gene, therefore specific *COMT* haplotypes could result in differential expression of the *COMT* enzyme when estrogenic levels vary. Furthermore, due to *COMT* playing a key role in the conjugation of catecholamines, *COMT* genotypes can also affect the circulation levels of estrogen (Liehr and Roy, 1990). This suggests a possible complex bidirectional interaction between *COMT* haplotypes and estrogenic hormones, which could have a potential impact on anxiety risk in a sex specific mode (Dempster *et al*., 2006).

Although we were unable to perform statistical analyses, the three male *COMT* haplotype C-A-C-G-A-A show an increase in scoring across subjective measures of anxiety, **Table 4.2**. For the Social Anxiety Questionnaire the C-A-C-G-A-A males report elevated scores, which are comparable to the high scoring observed across the female group. A similar observation was noted for the Spielberger State Trait Inventory, for both state and trait; where C-A-C-G-A-A males had only a slightly lower score than the female population score. This was found yet again for Beck Anxiety Inventory, with C-A-C-G-A-A males reporting similar scoring to the female population.

Table 4. 2 Anxiety scale scores for C-A-C-G-A-A males, the male and female population

	COMT SNP C-A-C-G-A-A (n=3 males)					Males (n=14)					Females (n=36)				
	mean	*std.dev.*	median	min -	max	mean	*std.dev.*	median	min -	max	mean	*std.dev.*	median	min -	max
Social Anxiety Questionnaire															
Speaking in public to authority	20.67	*1.15*	20	20 -	22	18.43	*4.09*	18	9 -	26	22.58	*5.64*	24	9 -	30
Interactions with the opposite sex	21.00	*2.65*	20	19 -	24	18.14	*4.49*	18	9 -	24	24.08	*4.78*	25	10 -	30
Assertive expression of annoyance/disgust/displeasure	21.67	*5.51*	19	18 -	28	17.07	*5.44*	18	10 -	30	19.56	*5.73*	20	7 -	29
Criticism/embarrassment	21.67	*5.51*	19	18 -	28	17.07	*5.44*	18	10 -	30	19.56	*5.73*	20	7 -	29
Interactions with strangers	22.00	*3.61*	21	19 -	26	17.50	*5.08*	18	7 -	24	20.58	*5.93*	22	6 -	30
Total score	107.00	*16.46*	98	97 -	126	88.21	*17.65*	93	48 -	116	106.36	*21.93*	105	50 -	146
Spielberger State Trait Inventory															
State anxiety	20.00	*1.00*	20	19 -	21	17.64	*5.21*	18	11 -	26	23.69	*7.53*	23	11 -	38
Trait anxiety	22.00	*6.08*	25	15 -	26	15.93	*3.60*	16	11 -	24	22.14	*5.84*	22	11 -	36
Beck Anxiety Inventory	41.33	*10.97*	35	35 -	54	36.36	*13.10*	32	21 -	63	43.31	*14.53*	40	23 -	78

Reporting on just the C-A-C-G-A-A males in comparison to the male and female population, the scoring for anxiety across all three scales are observed to be very similar between the C-A-C-G-A-A group and female population. The haplotype found for the three males, could therefore be possibly associated to elevated anxiety scoring.

Previous studies have linked low activity of the *COMT* rs4680 polymorphism to traits of anxiety, with homozygosity for the Met allele found to enhance the risk of PTSD (Enoch *et al.*, 2003; Olsson *et al*., 2005; Kolassa *et al*., 2010).The Met allele leads to an increase of dopamine levels in the prefrontal brain regions, as well as the amygdala and hippocampus (Chen *et al*., 2004; Guarraci *et al*., 2000). Additionally, dopamine reduces the inhibitory influence of the PFC on the amygdala, which causes a heightened input from sensory cortices and reduces affective control to sensory cues, with an increase in amygdala reactivity in response to emotional cues shown to be modulated by an increase in dopamine (Grace and Rosenkranz, 2000; Hariri *et al*.,2002). Bilder and colleagues (2004) suggested that an increase in the level of prefrontal dopamine levels could possibly cause impaired cognitive flexibility, which results in impaired disengagement of attention from adverse cues in surrounding environments (Bilder *et al*., 2004).

The impact *COMT* polymorphisms on disengagement of attention and response to emotional cues has been associated with anxiety disorders. A proposed model of attentional bias for anxiety by Cisler and Koster in 2010 suggests three main key components of this mechanism. The first is that detection of a threat mediates an association between enhanced engagements of an individual ad anxiety. The second is that attentional control mediates an association between delayed disengagement and anxiety. Lastly, emotional regulation mediates an association between attentional voidance and anxiety (Cisler and Koster, 2010).

4.2.2 The relationship between COMT and resilience

A number of studies have made use of GWAS for investigating the association between genetics and resilience, if any. Kang and co-authors looked at the genetic influence of *COMT* Val^{158}Met *(*rs4680), along with the brain-derived neurotropic factor (BDNF) Val^{66}Met for individual differences in resilience. Their findings suggest an association between *COMT* Val^{158}Met and resilience, modulated by BDNF Val^{66}Met in males. Males which had the *COMT* Met genotyped

had a significantly higher level of resilience than those with the Val/Val genotype, while no significant interactions were found amongst females (Kang *et al.*, 2013). In a more recent study which investigated resilience in the context of stress and candidate genes in the dopaminergic signalling pathway, *COMT* was found to be associated with stress resilience, modulated by both dopaminergic and serotonergic pathways. Additionally, variation in gene expression was observed to be associated with not just stress resilience but other psychological parameters of which anxiety and depression are included (Azadmarzabadi *et al*., 2018). These findings align with that of Armbuster and colleagues who reported the presence of the *COMT* met allele to result in stronger cortisol stress response in children, which suggest an interaction between *COMT* polymorphisms and stress that influence resilience (Amrbruster *et al*., 2012)

COMT has been identified as one of the most promising genes to be implicated in resilience, with studies linking rs4680 (Val^{158}Met) to resilience. Reported findings include Met allele carries to have a decreased state of emotional resilience against negative mood states and a positive correlation between hippocampus activation and resilience; where altered activation of the hippocampus is associated to the Met allele carriers (reduced activation) or Val homozygotes (increased activation) (Kolassa *et al*., 2010; van Rooij *et al*., 2016).

We again compare the three males with *COMT* haplotype C-A-C-G-A-A across subjective measures of resilience, **Table 4.3**. For Scale of Protective Factors, the male C-A-C-G-A-A scores for all sub dimensions but goal efficiency seem to be lower than both the male and female populations. This is also observed for the Adult Resilience measure, with the subscale 'Personal relationship with key individuals' being significantly lower, which carried over to the total score for the Adult Resilience measure, calculated by the sum of the total score across all sub scales. The Brief Resilience Scale was the only measure of resilience where the C-A-C-G-A-A males are found to have an elevated score in comparison to the male and female population.

Table 4. 3 Resilience scale scores for C-A-C-G-A-A males, the male and female population

	COMT SNP C-A-C-G-A-A (n=3 males)						Males (n=14)						Females (n=36)					
	mean	*std.dev.*	median	min	-	max	mean	*std.dev.*	median	min	-	max	mean	*std.dev.*	median	min	-	max
Scale of Protective Factors																		
Social skills	4.28	*0.86*	5	3	-	5	4.96	*1.35*	5	2	-	7	4.38	*1.16*	4	2	-	7
Social support	4.78	*1.11*	5	4	-	6	5.12	*1.14*	5	3	-	7	4.98	*0.99*	5	2	-	7
Goal efficiency	5.61	*1.17*	6	5	-	7	5.93	*0.75*	6	5	-	7	5.42	*0.91*	6	3	-	7
Planning prioritizing behaviour	4.11	*1.94*	4	2	-	6	4.90	*1.05*	5	4	-	7	5.11	*1.13*	5	3	-	7
Total score	18.78	*4.13*	17	16	-	24	20.91	*3.53*	21	15	-	27	19.89	*2.83*	21	15	-	25
Brief Resilience Scale	3.05	*0.69*	3	3	-	4	2.93	*0.54*	3	2	-	4	2.94	*0.50*	3	2	-	4
Adult Resilience Measure																		
Individual	4.15	*0.21*	4	4	-	4	4.25	*0.56*	4	3	-	5	4.05	*0.48*	4	3	-	5
Personal relationship with key individuals	3.57	*0.86*	4	3	-	4	4.32	*0.63*	4	3	-	5	4.19	*0.68*	4	2	-	5
Context/sense of belonging	3.70	*0.62*	4	3	-	4	4.01	*0.90*	4	2	-	5	3.90	*0.59*	4	2	-	5
Total score	107.67	*12.66*	103	98	-	122	117.07	*18.07*	121	74	-	139	112.86	*13.21*	115	81	-	134

The scoring observed across all three measures of resilience, with the exception of BRS showed the male C-A-C-G-A-A group to score much lower than both the male and female population, thus the haplotype cannot be considered to be indicative of resilience.

Few studies have investigated the impact of genetics on resilience, due to the larger number of resilience-related indicators that have been identified, with no gold standard for measurement of resilience defined (Windle *et al*., 2011; Rodriguez-Llanes *et al*., 2013). An overlap of indicators between psychological resilience and vulnerable phenotypes, especially in PTSD is a reflection of the aftermath of childhood trauma or adverse life events (Mattson *et al*., 2018). For this reason genetic based case control studies which investigates and compares those who have developed a psychiatric disorder following trauma exposure with those who have not developed a disorder may assist researchers in identifying genetic factors linked to resilience (Southwick and Charney, 2012).

4.2.3 The relationship between COMT and childhood trauma

For the longest time childhood trauma has been associated with the development of an array of psychiatric disorders, yet it is vital to understand the biological mechanisms which underline these disorders. Studies have shown that individuals which have the Val and Met variants of *COMT* differ in their levels of DA. This is due to the decrease of enzymatic action of *COMT* as a result of the Met substitution which leads to less breakdown of DA in the synapse and higher extracellular levels of DA (Chen *et al*., 2004). Being exposed to stressful or traumatic life events leads to an increase in DA activity, which can disturb the balance of DA. Therefore exposure to childhood trauma and any early life stress could potentially and differentially impact *COMT* Val/Val and Met carriers, specifically in brain regions where *COMT* is expressed, such as the PFC and hippocampus. Within the PFC and limbic structures of the human brain, a balance in DA tone is believed to be a requirement for the regulation of behaviour by the determination of relevancy of external environmental cues which results in behaviour being adapted accordingly (Antypa *et al*., 2013; Horvitz, 2000). Van Rooij and colleagues suggest this may explain why previous studies have found the impact of childhood trauma on inhibition-related hippocampal function to be dependent on the variation of *COMT* Val^{158}Met (van Rooij *et al*., 2016).

COMT has also been found to associate with childhood trauma alongside other features of psychiatric disorders and traits. Dissociation is defined as the failure of perceptual and emotional integration which has been linked to a number of psychiatric disorders, including bipolar disorder (BD) and has been further linked to childhood trauma. Due to a number of studies associating BD to childhood trauma and underlying genetics, Savitz and co-authors investigated if the relationship between childhood trauma and dissociation is influenced by *COMT* in 178 individuals and their relatives, which included the use of CTQ. Their findings showed that *COMT* rs4680 ($Val^{158}Met$) was significantly associated to total CTQ abuse scores to impact perceived dissociation. Individuals with the Met/Met genotype displayed a decrease in dissociation with an increase in childhood trauma, while those with the Val/Val genotype showed an increase in dissociation and exposure to higher levels of childhood trauma. These findings suggest the involvement of *COMT* (rs4680) in the facilitating of a relationship between childhood trauma and psychopathology (Savitz *et al*., 2008). In another study which tested the interaction between nine candidate genes, and childhood trauma in modulating anger related traits; the interaction between childhood trauma and *COMT* was found to influence the level of anger traits (Perroud *et al*., 2010).

For subjective measures of childhood trauma, the C-A-C-G-A-A males report an increase in score across all scales, **Table 4.4.** Observing scoring within the Bernstein Childhood Trauma Questionnaire sub dimensions, the C-A-C-G-A-A group report mean values higher for sexual abuse and adverse environment during childhood than the male and female population, while the range in reporting were similar. The C-A-C-G-A-A males report a significantly higher mean score for the Adverse Childhood Experience measure, with again almost identical range to male and female populations, which warrants for further investigation.

Table 4. 4 Childhood trauma scale scores for C-A-C-G-A-A males, the male and female population

	COMT SNP C-A-C-G-A-A (n=3 males)						Males (n=14)						Females (n=36)					
	mean	*std.dev.*	median	min	-	max	mean	*std.dev.*	median	min	-	max	mean	*std.dev.*	median	min	-	max
Bernstein Childhood Trauma Questionnaire																		
Emotional abuse	10.00	*5.20*	7	7	-	16	7.64	*3.08*	7	5	-	16	9.36	*4.64*	8	5	-	23
Physical abuse	7.00	*2.00*	7	5	-	9	7.57	*2.59*	7	5	-	14	6.14	*1.61*	6	5	-	13
Sexual abuse	9.67	*8.08*	5	5	-	19	5.93	*2.67*	5	5	-	15	6.25	*2.87*	5	5	-	17
Emotional neglect	9.67	*3.21*	11	6	-	12	9.50	*3.76*	9	5	-	18	9.06	*3.65*	9	5	-	16
Physical neglect	7.67	*1.53*	8	6	-	9	7.71	*2.52*	8	5	-	13	6.92	*2.25*	6	5	-	13
Total CTQ	44.00	*18.03*	39	29	-	64	38.36	*11.80*	37	28	-	74	37.72	*9.54*	37	25	-	65
Adverse Childhood Environment *	3.00	*3.61*	2	0	-	7	1.69	*2.18*	1	0	-	7	2.64	*2.63*	2	0	-	8

**For Adverse Childhood Environment, male group n = 13 and female group n= 33.*

The elevated mean scores across childhood trauma measures for C-A-C-G-A-A males is of significance in comparison to the male and female population, with a large difference in reporting. Considering the similar trend observed across the anxiety measures, and the reported association between anxiety and childhood trauma in previous studies, the C-A-C-G-A-A haplotype for *COMT* needs to be further investigated.

Exposure to childhood trauma is suggested to act as a life stressor resulting in cascade of hormonal changes and physiological reactions which lead to overstimulation of neurons during critical periods. This series of events results in modification of neural function and structure (Teicher, 2002). Although brain development is suggested to be modified by exposure to childhood trauma, it is essentially directed by gene interaction, with genetic predisposition influencing the impact of environmental adversity on both brain function and structure. Therefore the interaction between childhood trauma and genetics is suggested to lead into the development of psychiatric symptoms during adolescent life (Caspi *et al*., 2003).

4.3 Other candidate genes associated to anxiety, resilience and childhood trauma

Aside from *COMT*, there are a number of other genes investigated in candidate genes and GWAS studies. In GAD, the genes for monoamine oxidase A (MAOA), BDNF, the short allele of the serotonin transporter-linked polymorphic regions (5-HTTLPR) in the serotonin transporter gene (SLC6A4) and neuropeptide Y (NPY) are just a few that have been identified by research (You *et al.*, 2005; Amstadter *et al.*, 2010; Molina *et al*., 2011; Tadic *et al*., 2003; Zhang *et al*., 2017). However, even with these genes being identified as target genes for GAD specifically, majority of psychiatric disorders are thought to be polygenic and not limited to a single gene (Duncan *et al*., 2019).Recently, five loci have been identified in individuals of European ancestry from the UK Biobank to be associated with anxiety disorders The identified loci are rs10809485, rs1187280 in neurotrophic receptor tyrosine kinase 2 (NTRK2), rs3807866 of transmembrane protein 106B (TMEM106B), rs2861139 on chromosome 5, and rs4855559 in the myosin heavy chain 15 (MYH15) gene (Purves *et al*., 2019). All have been associated to a range of anxiety disorders

which included GAD and PD, supporting the idea that psychiatric disorders are polygenetic. This warrants for future studies to not limit the gene-psychiatric disorder association to singular genes.

A recent GWAS study on resilience identified three new candidate genes found to be implicated in resilience as well as PTSD; namely doublecortin-like kinase (DCLK2), Kelch-like family member 36 (KLHL36) and Solute Carrier Family 15 Member 5(SLC15A5) (Maul *et al*., 2019). In Stein's 2019 GWAS study on psychological resilience in 11,492 U.S soldiers, four SNPs on chromosome 4, upstream of DCLK2 was found to promote the survival and regeneration of injured neurons, together with KLHL36 which reported significant association to resilience. In addition, SLC15A5 on chromosome 12 was identified as a polygenic risk gene in individuals which were exposed to high levels of stress and showed outcome based resilience (Stein *et al*., 2019). An earlier study reported variation in two other genes; CRH receptor (CRHR1) and FK506-binding protein 51 (FKB5) to lead to the development of resilience factors when exposure to childhood abuse is combined with biological risk factors (Gillespie *et al*., 2009).

While childhood trauma is a known risk factor across a range of psychiatric conditions, the genetic liability is not well understood. To date, no single study has investigated genes linked solely to childhood trauma, with all studies linking childhood trauma and a specific psychiatric condition or behavioural outcome, in the context of epigenetics. However, there is a number of studies which report changes in DNA methylation as a result of childhood trauma exposure (Gesellschaft, 2012; Peng *et al*., 2018). Considering epigenetic modifications, such as DNA methylation, in context of childhood trauma, Jiang and collagues reported a number of stress genes associated to early trauma exposure. The genes FKB5, MAO, nuclear receptor subfamily 3 group C member 1 (NR3C1), 5-serotonin 3A receptor (HTR3A), 5-HTTLPR and SLC6A4 are just a few identified stress genes with SNPs implicated in childhood trauma (Jiang *et al*., 2019).

4.4 Conclusion

The aims of the study was to develop a multiplex for *COMT* SNPs, while identifying the respective SNPs associated to anxiety, resilience and childhood trauma in a South African sample. Several aims were set out; namely to develop a multiplex assay for genotyping several *COMT* SNPs with the purpose of identifying a possible haplotype linked to anxiety, resilience and childhood trauma;

to investigate the prevalence of anxiety, resilience and childhood as functional behavioural categories in the full South African sample group; and the role of sex through established self-report measures and respective normative data; and lastly to investigate the correlations between anxiety, resilience and childhood trauma as a multidimensional construct in both the full South African sample and between sexes

To achieve the first aim, Chapter 2, several objectives were set, which were to design *COMT* SNP primers and an optimized multiplex, followed by an optimized SNaPshot assay for genotyping of *COMT* SNPs. Primers for SBE were successfully designed, yet the design of a single multiplex reaction consisting of all six *COMT* SNP primers was unsuccessful, which resulted in several duplex PCR reactions followed by later pooling of products for SNaPshot. Due to the large binding region of *COMT*, designing primers which are allele specific and incorporating all into a single multiplex reaction requires further time and careful optimization. Genotyping of the full sample set was not achieved due to time constraints and limited laboratory equipment, yet the sub set of 11 samples which were used for optimization and testing of SNaPshot proved to be an efficient and accurate method for calling SNP allele's easily.

The second aim, Chapter 3, was to investigate the relationship between three functional behavioural categories; namely anxiety, resilience and childhood trauma, while comparing scoring of the sample group with normative data. This followed with investigating differentiation between sexes through established self-report scales and respective normative data; and investigating correlations between anxiety, resilience and childhood trauma in both the full sample and between sexes. This was successfully achieved, showing correlation between anxiety, childhood trauma and resilience. Sex differences were not apparent in the context of resilience and childhood trauma, yet in anxiety females reported higher scoring.

The third aim, Chapter 4, was to draw together the empirical data, while reviewing the literature and exploring the data where both *COMT* SNP and self-report data were collected for measures of anxiety, resilience, and childhood trauma for male and female. Despite the limitation of data, this was successfully achieved, with a comparative review of published literature suggesting association between all three functional behavioural categories as a multidimensional construct.

Since almost all individuals are undergo a stressful or traumatic event at some point in life, understanding why some are able to navigate through these events is important in maintaining mental health in society. *COMT* has been proven to be one of many ideal candidate genes associated with environmental adversity while increasing risk for the development of psychiatric disorders such as anxiety as well as enhanced resiliency. Aside from the treatment and diagnostics of neuropsychiatric disorders, identification of possible risk factors are essential for the promotion of better mental health in society. It is for this reason that an improved understanding of the genetic mechanisms which underline anxiety, resilience and childhood trauma will assist in improved treatment and prevent strategies

The need for genetic studies, coupled with self-report measures as tools for investigating the relationship and association between anxiety, childhood trauma and resilience is supported by the abundance of research which has linked each of these functional behavioural categories to a variety of candidate genes separately. Each of these are complex and multidimensional constructs which have a direct relationship with one another, yet are influenced by a range of internal and external factors of which epigenetics is included.

Future studies could incorporate the identification of candidate genes linked to each behavioural category through GWAS on large sample sets of mixed ethnicity to finally provide a deep and better understanding of how our genetics impact and influence psychiatric disorders, resilience and childhood trauma exposure. The use of an array of SNPs which support the function of candidate genes associated to each of the functional behavioural categories is fundamental in providing a detailed understanding of anxiety, resilience and childhood trauma as a multidimensional construct.

REFERENCES

Agid, O., Kohn, Y., Lerer, B. (2000) 'Environmental stress and psychiatric illness', Biomedicine and Pharmacotherapy, 54(3), pp. 135–141.

Ahmad, Nor; Khairani, Ahmad & Aman, Rahimi. (2018). Assessing Resilience among Malaysian University Undergraduates.

Ahmed, R., Seedat, M., Van Niekerk, A., Bulbulia, S. (2004). Discerning community resilience in disadvantaged communities in the context of violence and injury prevention. South African Journal of Psychology, 34(3), 386– 408.

Al Odhayani A, Watson WJ, Watson L (2013) Behavioural consequences of child abuse. Can Fam Physician.; 59:831–836

Altinyazar, V., Gunderici, A., Tinaz, E.; Kirci, C. (2015). No association of Catechol-O-Methyltransferase (COMT) gene haplotypes in patients with schizophrenia in a Turkish sample. Bulletin of Clinical Psychopharmacology, 25, 129–135.

Amat, S., Subhan, M., Jaafar, W. M. W., Mahmud, Z.; Johari, K. S. K. (2014). Evaluation and Psychometric Status of the Brief Resilience Scale in a Sample of Malaysian International Students. Asian Social Science, 10(18), 240-245.

American Psychiatric Association. (2013).Diagnostic and statistical manual of mental disorders (5th Ed.). Arlington, VA: Author.

Anda, R. F., Felitti, V. J., Bremner, J. D., Walker, J. D., Whitfield, C., Perry, B. D., Dube, S. R., Giles, W. H. (2006). The enduring effects of abuse and related adverse experiences in childhood. A convergence of evidence from neurobiology and epidemiology. European archives of psychiatry and clinical neuroscience, 256(3), 174–186.

Angers, B., Castonguay, E., Massicotte, R. (2010) Environmentally induced phenotypes and DNA methylation: how to deal with unpredictable conditions until the next generation and after. Molecular Ecology, 19: 1283-1295.

Armbruster D., Mueller A., Strobel A., Lesch K. P., Brocke B., Kirschbaum C. (2012). Children under stress - COMT genotype and stressful life events predict cortisol increase in an acute social stress paradigm. Int. J. Neuropsychopharmacol. 15, 1229–1239

Asher, Maya; Aderka, Idan. (2018). Gender differences in social anxiety disorder. Journal of Clinical Psychology. 74. 10.

Azadmarzabadi, E., Haghighatfard, A., Mohammadi, A. (2018), Low resilience to stress is associated with candidate gene expression alterations in the dopaminergic signalling pathway. Psychogeriatrics, 18: 190-201

Baglivio, M.T., Epps, N., Swartz, K., Huq, M.S., Sheer, A., Hardt, N.S. (2014) The Prevalence of Adverse Childhood Experiences (ACE) in the Lives of Juvenile Offenders. Journal of Juvenil Justice. Vol (3) Issue 2

Ballenger-Browning, K., Johnson, D.C. (2010). Key Facts on Resilience.

Baños, R. M., Botella, C., Quero, S., Medina, P. (2007). The social phobia and anxiety inventory: Psychometric properties in a spanish sample. Psychological Reports, 100, 441–450.

Barnett, J.H., Heron, J., Goldman, D., Jones, P.B., Xu, K. (2009). Effects of catechol-O-methyltransferase on normal variation in the cognitive function of children. American Journal of Psychiatry. 166(8):909–916.

Bartlett, Jessica, Steber, Kate. (2019). How to Implement Trauma-informed Care to Build Resilience to Childhood Trauma -Child Trends.

Bateman, C. (2015). The risky lives of South Africa's children: Why so many die or are traumatised. South African Medical Journal. 105(3):170–171.

Baumann, C., Klauke, B., Weber, H., Domschke, K., Zwanzger, P., Pauli, P., Deckert, J.,Reif, A. (2013) The interaction of early life experiences with *COMT*val158met affects anxiety sensitivity. Genes, Brain and Behavior, 12: 821-829.

Beck, A. T., Epstein, N., Brown, G., Steer, R. A. (1988). An inventory for measuring clinical anxiety: Psychometric properties. Journal of Consulting and Clinical Psychology, 56(6), 893.

Beck, A. T., Steer, R. A. (1993a). Manual for the Beck Anxiety Inventory.San Antonio, TX:The Psychological Corporation.

Bernegger, A., Kienesberger, K., Carlberg, L., Swoboda, P., Ludwig, B., Koller, R., Inaner, M., Zotter, M., Kapusta, N., Aigner, M., Haslacher, H., Kasper, Schosser, A. (2018). The Impact of COMT and Childhood Maltreatment on Suicidal Behaviour in Affective Disorders. Scientific Reports, 8(1).

Bernstein, D.P., Fink, L. (1998). Childhood Trauma Questionnaire: A retrospective self-report manual San Antonio, TX: The Psychological Corporation.

Betancourt, T. S., Meyers-Ohki, S., Stulac, S. N., Elizabeth Barrera, A., Mushashi, C., Beardslee, W. R. (2011). Nothing can defeat combined hands (Abashize hamwe ntakibananira): Protective International Journal of Adolescence and Youth 253 processes and resilience in Rwandan children and families affected by HIV/AIDS. Social Science & Medicine, 73(5), 693– 701.

Bichard, S. (2000). Child Development for Child Care and Protection Workers. Child Psychology and Psychiatry Review, 5(1), pp.46–48.

Biederman, J., Faraone, S. V., Hirshfeld-Becker, D.R., Friedman, D., Robin, J.A.,Rosenbaum, J.F. (2001). Patterns of psychopathology and dysfunction in high-risk children of parents with panic disorder and major depression. American Journal of Psychiatry. 158(1):49–57

Bilder, RM, Volavka, J, Lachman, HM, Grace, AA (2004) 'The catechol-O-methyltransferase polymorphism: Relations to the tonic-phasic dopamine hypothesis and neuropsychiatric phenotypes', Neuropsychopharmacology, vol. 29, no. 11, pp. 1943-1961

Brooks, M., Lowe, M., Graham-Kevan, N., Robinson, S. (2016).Posttraumatic growth in students, crime survivors and trauma workers exposed to adversity.Pers. Individ. Dif.98, 199–207.

Bruijnen, C., Young, S., Marx, M., Seedat, S. (2019). Social anxiety disorder and childhood trauma in the context of anxiety (behavioural inhibition), impulsivity (behavioural activation) and quality of life. South African Journal of Psychiatry, 25, 7

Boscarino JA, Adams RE, Figley CR. (2011) Mental health service use after the World Trade Center disaster: Utilization trends and comparative effectiveness. The Journal of Nervous and Mental Disease. 199(2):91–99

Bourdon, K.H., Boyd, J.H., Rae, D.S., Burns, B.J., Thompson, J.W., Locke, B.Z. (1988). Gender differences in phobias: Results of the ECA community survey. Journal of Anxiety Disorders. 2(3):227–241.

Butler, J. M. (2012). Single Nucleotide Polymorphisms and Applications. Advanced Topics in Forensic DNA Typing, 347–369.

Butler JM, Coble MD, Vallone PM. (2007) STRs vs. SNPs: thoughts on the future of forensic DNA testing.Forensic Sci Med Pathol. 3(3):200-205.

Caballo, V.E., Salazar, I.C., Irurtia, M.J., Arias, B., Hofmann, S.G. (2010). Measuring social anxiety in 11 Countries: Development and validation of the social anxiety questionnaire for adults. European Journal of Psychological Assessment. 26(2):95–107.

Caballo, V. E., Salazar, I. C., Irurtia, M. J., Arias, B., Hofmann, S. G. (2012). The multidimensional nature and multicultural validity of a new measure of social anxiety the Social Anxiety Questionnaire for Adults (SAQ-A30). Behavior Therapy, 43, 313-328.

Caballo, V.E., Salazar, I.C., Irurtia, M.J., Arias, B., Nobre, L. (2013). The assessment of social anxiety through five self-report measures, LSAS-SR, SPAI, SPIN, SPS, and SIAS: A critical analysis of their factor structure.

Calitz, F.J.W., Heckler, H., Taute, C.E., Krüger, G.H.J., Wet, D. de, Merwe, L. van der., Raubenheimer, J.E. (2012). Profile of stress factors associated with mental disorders in children and adolescents referred for evaluation and treatment to the Free State Psychiatric Complex, 2007. South African Journal of Psychiatry, 18(2), pp.61–67.

Campbell-Sills, L., Cohan, S.L., Stein, M.B. (2006). Relationship of resilience to personality, coping, and psychiatric symptoms in young adults. Behaviour Research and Therapy. 44(4):585–599.

Carrey, N.; Ungar, M. (2007). Resilience. Monograph for the Child and Adolescent Psychiatry Clinics of North America, 162, 271– 540.

Carver, C. S.; Scheier, M. F. (1998). On the self-regulation of behavior. New York: Cambridge University Press.

Caspi A, Sugden K, Moffitt TE, Taylor A, Craig IW, Harrington H, McClay J, Mill J, Martin J, Braithwaite A, Poulton R. (2003) Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. Science.301:386–389

Chambers CD, Garavan H, Bellgrove MA. (2009) Insights into the neural basis of response inhibition from cognitive and clinical neuroscience. Neurosci Biobehav Rev. 33(5):631–46.

Charak, R., de Jong, J.T.V.M., Berckmoes, L.H., Ndayisaba, H., Reis, R. (2017). Assessing the factor structure of the Childhood Trauma Questionnaire, and cumulative effect of abuse and neglect on mental health among adolescents in conflict-affected Burundi. Child Abuse and Neglect. 72:383–392.

Charney, D. S. (2003). The psychobiology of resilience and vulnerability to anxiety disorders: implications for prevention and treatment. Dialogues in clinical neuroscience, 5(3), 207–221.

Chen, J., Lipska, B.K., Halim, N., Ma, Q.D., Matsumoto, M., Melhem, S., Kolachana, B.S., Hyde, T.M., Herman, M.M., Apud, J., Egan, M.F., Kleinman, J.E.,Weinberger, D.R. (2004). Functional Analysis of Genetic Variation in Catechol-O-Methyltransferase (*COMT*): Effects on mRNA, Protein, and Enzyme Activity in Postmortem Human Brain.*American* Journal of Human Genetics, 75(5), pp.807–821.

Choi J, Jeong B, Rohan ML, Polcari AM, Teicher MH. (2009) Preliminary evidence for white matter tract abnormalities in young adults exposed to parental verbal abuse. Biol Psychiatry.65:227–234.

Chorpita, B.F., Taylor, A.A. (2002). Behavioral Assessment of Anxiety Disorders. Practitioner's Guide to Empirically Based Measures of Anxiety, pp.19–24.

Cisler, J.M., Koster, E.H.W. (2010). Mechanisms of attentional biases towards threat in anxiety disorders: An integrative review. Clinical Psychology Review, 30(2), pp.203–216.

Cisler, J. M., Olatunji, B. O., Feldner, M. T., Forsyth, J. P. (2010). Emotion Regulation and the Anxiety Disorders: An Integrative Review. Journal of psychopathology and behavioral assessment, 32(1), 68–82.

Cohen, L., Ferguson, C., Harms, C., Pooley, J.A., Tomlinson, S. (2011). Family systems and mental health issues: A resilience approach. Journal of Social Work Practice. 25(1):109–125.

Cohen, P., Kasen, S., Chen, H., Hartmark, C. & Gordon, K. (2003). Variations in patterns of developmental transitions in the emerging adulthood period. Developmental Psychology, • 39(4), 657-669.

Collin-Vézina, D., Coleman, K., Milne, L., Sell, J., Daigneault, I. (2011). Trauma Experiences, Maltreatment-Related Impairments, and Resilience Among Child Welfare Youth in Residential Care. International Journal of Mental Health and Addiction. 9(5):577–589.

Collishaw, S., Pickles, A., Messer, J., Rutter, M., Shearer, C., Maughan, B. (2007). Resilience to adult psychopathology following childhood maltreatment: evidence from a community sample. Child Abuse Neglect. 31, 211–229.

Connor, K.M., Davidson, J.R.T. (2003). Development of a new Resilience scale: The Connor-Davidson Resilience scale (CD-RISC). Depression and Anxiety. 18(2):76–82.

Cox, B.J., Cohen, E., Direnfeld, D.M. & Swinson, R.P. (1996). Does the beck anxiety inventory measure anything beyond panic attack symptoms? Behaviour Research and Therapy. 34(11–12):949–954.

Creamer, M., Foran, J., Bell, R. (1995). The Beck Anxiety Inventory in a non-clinical sample. Behaviour Research and Therapy. 33(4):477–485.

Cullerton-Sen, C., Cassidy, A.R., Murray-Close, D., Cicchetti, D., Crick, N.R., Rogosch, F.A. (2008). Childhood maltreatment and the development of relational and physical aggression: The importance of a gender-informed approach. Child Development. 79(6):1736–1751.

Danese, A., McEwen, B. S. (2012). Adverse childhood experiences, allostasis, allostatic load, and age-related disease. Physiology & behavior, 106(1), 29–39.

Dempster, E., Toulopoulou, T., McDonald, C., Bramon, E., Walshe, M., Filbey, F., Wickham, H., Sham, P.C., Murray, R.M., Collier, D.A. (2005), Association between BDNF val66 met genotype and episodic memory. Am. J. Med. Genet., 134B: 73-75

Domschke, K., Freitag, C.M., Kuhlenbäumer, G., Schirmacher, A., Sand, P., Nyhuis, P., Jacob, C., Fritze, J,Franke, P.,Rietschel, M.,Garritsen, H.S.,Fimmers, R.,Nöthe, M.M.,Lesch, K.P.,Stögbauer, F.,Deckert, J. (2004) Association of the functional V158M catechol-O-methyl-transferase polymorphism with panic disorder in women. Int J Neuropsychopharmacol 7, 183–188.

Domschke, K., Tidow, N., Kuithan, H., Schwarte, K., Klauke, B., Ambrée, O., Reif, A., Schmidt, H., Arolt, V., Kersting, A., Zwanzger, P.; Deckert, J. (2012) Monoamine oxidase A gene hypomethylation–a risk factor for panic disorder? Int J Neuropsychopharmacol 15, 1217–1228.

Drabant, E.M., Hariri, A.R., Meyer-Lindenberg, A., Munoz, K.E., Mattay, V.S., Kolachana, B.S. (2006) Catechol-O-methyltransferase Val158Met genotype and neural mechanisms related to affective arousal and regulation.Arch Gen Psychiatry 63, 1396–1406.

Dubow, E.F., Boxer, P., Huesmann, L.R., Shikaki, K., Landau, S., Gvirsman, S.D., Ginges, J. (2010). Exposure to conflict and violence across contexts: Relations to adjustment among palestinian children. Journal of Clinical Child and Adolescent Psychology. 39(1):103–116.

Dudley K. J., Li X., Kobor M. S., Kippin T. E., Bredy T. W. (2011). Epigenetic mechanisms mediating vulnerability and resilience to psychiatric disorders. Neurosci. Biobehav. Rev. 35, 1544–1551

Duncan, G.J., Brooks-Gunn, J., Klebanov, P.K. (1994). Economic Deprivation and Early Childhood Development. Child Development. 65(2):296–318.

Duncan, L., Shen, H., Gelaye, B., Meijsen, J., Ressler, K., Feldman, M., Peterson, R., Domigue, B (2019) Analysis of polygenic risk score usage and performance in diverse human populations. Nat Commun 10, 328.

Dymond, S., Roche, B. (2009). A contemporary behavior analysis of anxiety and avoidance. The Behavior analyst, 32(1), 7–27.

Edwards R, Rodriguez-Brito B, Wegley L, Haynes M, Breitbart M, Peterson D, Saar M, Alexander S, Alexander EC, Rohwer F (2006) Using pyrosequencing to shed light on deep mine microbial ecology. BMC Genomics 7: 57–70.

Eisenhofer G, Kopin IJ, Goldstein DS. (2004) Catecholamine metabolism: a contemporary view with implications for physiology and medicine.Pharmacol Rev.56 (3):331-49

Ejaz, B., Muazzam, A., Anjum, A., Pollock, G., Nawaz, R. (2020). Measuring the scale and scope of social anxiety among students in Pakistani higher education institutions: An alternative social anxiety scale. Sustainability (Switzerland). 12(6):2164.

Endler, N. S., Kocovski, N. L. (2001). State and trait anxiety revisited. Anxiety Disorders, 15, 231-245.

Enoch, M.A.,Xu, K.,Ferro, E.,Harris, C.R, Goldman, D.(2003) Genetic origins of anxiety in women: a role for a functional catechol-O-methyltransferase polymorphism.Psychiatr Genet.(13),33–41.

Enoch, M. A., Waheed, J. F., Harris, C. R., Albaugh, B., Goldman, D. (2009). COMT Val158Met and cognition: main effects and interaction with educational attainment.Genes, brain, and behavior, 8(1), 36–42.

Etkin A, Wager TD. (2007) Functional neuroimaging of anxiety: a meta-analysis of emotional processing in PTSD, social anxiety disorder, and specific phobia. Am J Psychiatry. 164(10):1476-88.

Felitti, V.J., Anda, R.F., Nordenberg, D., Williamson, D.F., Spitz, A.M., Edwards, V., Koss, M.P. & Marks, J.S. (1998). Relationship of childhood abuse and household dysfunction to many

of the leading causes of death in adults: The adverse childhood experiences (ACE) study. American Journal of Preventive Medicine. 14(4):245–258.

Fossion, P., Leys, C., Kempenaers, C., Braun, S., Verbanck, P., Linkowski, P. (2014). Disentangling Sense of Coherence and Resilience in case of multiple traumas. Journal of Affective Disorders. 160:21–26.

Friborg, O., Barlaug, D., Martinussen, M., Rosenvinge, J.H., Hjemdal, O (2005). Resilience in relation to personality and intelligence. International Journal of Methods in Psychiatric Research. 14(1):29–42.

Friborg, O., Hjemdal, O., Rosenvinge, J.H., Martinussen, M. (2003). A new rating scale for adult resilience: What are the central protective resources behind healthy adjustment? International Journal of Methods in Psychiatric Research. 12(2):65–76.

Funke, B., Malhotra, A. K., Finn, C. T., Plocik, A. M., Lake, S. L., Lencz, T., DeRosse, P., Kane, J. M., Kucherlapati, R. (2005). COMT genetic variation confers risk for psychotic and affective disorders: a case control study. Behavioral and brain functions. BBF, 1, 19.

Fydrich, T., Dowdall, D., Chambless, D.L. (1992). Reliability and validity of the beck anxiety inventory. Journal of Anxiety Disorders. 6(1):55–61.

Gardner, T.W., Dishion, T.J., Connell, A.M. (2008). Adolescent self-regulation as resilience: Resistance to antisocial behavior within the deviant peer context. Journal of Abnormal Child Psychology. 36(2):273–284.

Gesellschaft, M.C (2012) Childhood trauma leaves mark on DNA of some victims: Gene-environment interaction causes lifelong dysregulation of stress hormones. ScienceDaily.

Gibson, K., Swartz, L., Sandenbergh, R. (2002). Counselling and Coping. Cape Town: Oxford University Press.

Gillespie, C. F., Phifer, J., Bradley, B., Ressler, K. J. (2009). Risk and resilience: genetic and environmental influences on development of the stress response. Depression and anxiety, 26(11).

Gregorowski, C., Seedat, S. (2013). Addressing childhood trauma in a developmental context. Journal of Child & Adolescent Mental Health, 25(2), pp.105–118.

Grillon, C. (2002). Startle reactivity and anxiety disorders: aversive conditioning, context, and neurobiology. Biol. Psychiatry 52, 958–975

Groth-Marat, G (2009) The Handbook of Psychological Assessment. Third edition

Gee, D. G., & Casey, B. J. (2015). The impact of developmental timing for stress and recovery. Neurobiology of Stress, 1, 184–194.

Glusman, G., Cox, H.C. and Roach, J.C. (2014). Whole-genome haplotyping approaches and genomic medicine.Genome Medicine, 6(9).

Goldberg, T.E., Egan, M.F., Gscheidle, T., Coppola, R., Weickert, T., Kolachana, B.S., Goldman, D., Weinberger, D.R. (2003). Executive subprocesses in working memory: Relationship to catechol-O-methyltransferase Val158Met genotype and schizophrenia. Archives of General Psychiatry. 60(9):889–896.

Goldstein D. S. (2010). Catecholamines 101. Clinical autonomic research. Official journal of the Clinical Autonomic Research Society, 20(6), 331–352.

Gottschalk, M. G., Domschke, K. (2017). Genetics of generalized anxiety disorder and related traits. Dialogues in clinical neuroscience, 19(2), 159–168.

Grace, A.A., Rosenkranz, J.A. (2002). Regulation of conditioned responses of basolateral amygdala neurons. Physiology and Behavior. 77(4–5):489–493.

Graham, R., Liew, M., Meadows, C., Lyon, E., Wittwer, C.T. (2005). Distinguishing different DNA heterozygotes by high-resolution melting. Clinical Chemistry. 51(7):1295–1298.

Gruss, L. F., Langaee, T., Keil, A. (2016). The role of the COMT val158met polymorphism in mediating aversive learning in visual cortex.NeuroImage, 125, 633–642.

Guarraci, F.A., Frohardt, R.J., Falls, W.A., Kapp, B.S. (2000). The effects of intra-amygdaloid infusions of a D2 dopamine receptor antagonist on Pavlovian fear conditioning. Behavioral Neuroscience. 114(3).

Halfaker, D.A., Akeson, S.T., Hathcock, D.R., Mattson, C., Wunderlich, T.L. (2011). Psychological Aspects of Pain. In Pain Procedures in Clinical Practice. Elsevier Inc. 13–22.

Hall, K. T., Loscalzo, J., Kaptchuk, T. J. (2019). Systems pharmacogenomics - gene, disease, drug and placebo interactions: a case study in *COMT*. Pharmacogenomics, 20(7), 529–551.

Hantsoo, L., Epperson, C.N. (2017). Anxiety Disorders Among Women: A Female Lifespan Approach. Focus. 15(2):162–172.

Hariri, H.R., Mattay, V.S., Tessitore, A., Fera, F., Smith, W.G., Weinberger, D.R. (2002) Dextroamphetamine modulates the response of the human amygdala. Neuropsychopharmacology 27, 1036–1040.

Herman, A. A., Stein, D. J., Seedat, S., Heeringa, S. G., Moomal, H., Williams, D. R. (2009). The South African Stress and Health (SASH) study: 12-month and lifetime prevalence of common mental disorders.South African medical journal,99(5 Pt 2), 339–344.

Hernandez, A., Gallardo-Pujol, D., Pereda, N., Arntz, A., Bernstein, D.P., Gaviria, A.M., Labad, A., Valero, J. and Gutiérrez-Zotes, J.A. (2013). Initial validation of the Spanish childhood trauma questionnaire-short form: factor structure, reliability and association with parenting. Journal of Interpersonal Violence, 28(7), pp.1498–1518

Herzog, J. I., Schmahl, C. (2018). Adverse Childhood Experiences and the Consequences on Neurobiological, Psychosocial, and Somatic Conditions Across the Lifespan. Frontiers in psychiatry, 9, 420.

Hettema JM., Prescott CA., Myers JM., Neale MC., Kendler KS. (2005) The structure of genetic and environmental risk factors for anxiety disorders in men and women. Arch Gen Psychiatry.62(2):182–189

Hettema JM, Annas P, Neale MC, Fredrikson M, Kendler KS. (2008) The genetic covariation between fear conditioning and self-report fears.Biol Psychiatry. 63:587–593

Higgins, F. (2015). Molecular Genetic Analyses of Antipsychotic Pharmacogenes in a South African First Episode Schizophrenia Cohort. Stellenbosch, Stellenbosch University.

Hirai, M., Vernon, L.L., Clum, G.A., Skidmore, S.T. (2011). Psychometric properties and administration measurement invariance of social phobia symptom measures: Paper-pencil vs. internet administrations. Journal of Psychopathology and Behavioral Assessment. 33(4):470–479.

Hogarth, L., Hardy, L., Mathew, A. R., Hitsman, B. (2018). Negative mood-induced alcohol-seeking is greater in young adults who report depression symptoms, drinking to cope, and subjective reactivity. Experimental and clinical psychopharmacology, 26(2), 138–146.

Horn, S. R., Charney, D. S., Feder, A. (2016). Understanding resilience: New approaches for preventing and treating PTSD. Experimental neurology, 284(Pt B), 119–132.

Hovens, J.G.F.M., Giltay, E.J., Wiersma, J.E., Spinhoven, P., Penninx, B.W.J.H., Zitman, F.G. (2012). Impact of childhood life events and trauma on the course of depressive and anxiety disorders. Acta Psychiatrica Scandinavica. 126(3):198–207.

Hughes, K., Bellis, M.A., Hardcastle, K.A., Sethi, D., Butchart, A., Mikton, C., Jones, L., Dunne, M.P. (2017). The effect of multiple adverse childhood experiences on health: a systematic review and meta-analysis. The Lancet Public Health. 2(8):e356–e366.

Jain, S., Cohen, A. (2013). Behavioral adaptation among youth exposed to community violence: nA longitudinal multidisciplinary study of family, peer and neighborhood-level protective factors.Prevention Science, 14, 606–617.

Jiang, S., Postovit, L., Cattaneo, A., Binder, E.B., Aitchison, K.J. (2019). Epigenetic Modifications in Stress Response Genes Associated With Childhood Trauma. Frontiers in Psychiatry, 10.

Jones, L. (2019). Resilience isn't the same for all: Comparing subjective and objective approaches to resilience measurement. Wiley Interdisciplinary Reviews: Climate Change. 10(1):e552.

Julian, L. J. (2011). Measures of anxiety: State-Trait Anxiety Inventory (STAI), Beck Anxiety Inventory (BAI), and Hospital Anxiety and Depression Scale-Anxiety (HADS-A).Arthritis care & research. 63 Suppl 11(0 11), S467–S472.

Kabacoff, R. I., Segal, D. L., Hersen, M., Van Hasselt, V. B. (1997). Psychometric properties and diagnostic utility of the Beck Anxiety Inventory and the State–Trait Anxiety Inventory with older adult psychiatric outpatients. Journal of Anxiety Disorders, 11, 33–47.

Kalmakis, K.A.,Chandler, G.E. (2015). Health consequences of adverse childhood experiences: A systematic review. Journal of the American Association of Nurse Practitioners. 27(8):457–465.

Kamal, S., Lappin, S.L. (2019).Biochemistry, Catecholamine Degradation. PubMed.

Karos, K., Niederstrasser, N., Abidi, L., Bernstein, D.P., Bader, K. (2014). Factor structure, reliability, and known groups validity of the German version of the Childhood Trauma Questionnaire (short-form) in Swiss patients and nonpatients. Journal of Child Sexual Abuse. 23(4):418–430.

Kascakova, N., Furstova, J., Hasto, J., Madarasova Geckova, A., Tavel, P. (2020). The Unholy Trinity: Childhood Trauma, Adulthood Anxiety, and Long-Term Pain. International Journal of Environmental Research and Public Health, 17(2), p.414.

Kang, J. I., Kim, S. J., Song, Y. Y., Namkoong, K., An, S. K. (2013). Genetic influence of *COMT* and BDNF gene polymorphisms on resilience in healthy college students. Neuropsychobiology, 68(3), 174–180.

Kessler, R.C., Stein, M.B., Berglund, P. (1998) Social phobia subtypes in the National Comorbidity Survey. American Journal of Psychiatry. 155(5):613–619.

Kim SJ, Kim YS, Kim SY, Lee HS, Kim CH. (2006) An Association Study of Catechol-O-Methyltransferase and Monoamine Oxidase A Polymorphisms and Personality Traits in Koreans. Neurosci.Lett. Jun 19. 401(1–2):154–158

Kisely, S., Abajobir, A., Mills, R., Strathearn, L., Clavarino, A., Najman, J. (2018). Child maltreatment and mental health problems in adulthood: Birth cohort study. The British Journal of Psychiatry, 213(6), 698-703.

Kolassa I-T, Kolassa S, Ertl V, Papassotiropoulos A, De Quervain DJ. (2010) The risk of posttraumatic stress disorder after trauma depends on traumatic load and the catechol-O-methyltransferase Val158Met polymorphism. Biol Psychiatry. 67(4):304–8.

Kroska, E. B., Miller, M. L., Roche, A. I., Kroska, S. K., O'Hara, M. W. (2018). Effects of traumatic experiences on obsessive-compulsive and internalizing symptoms: The role of avoidance and mindfulness. Journal of affective disorders, 225, 326–336.

Kwon, Aeran, Dongil & Kim, Yourim & Jin, Min., Lee, Seung-Hwan. (2020). Interaction between catechol-O-methyltransferase polymorphism and childhood trauma in suicidal ideation of patients with post-traumatic stress disorder. Brain and Behavior.

Lachman HM, Papolos DF, Saito T, Yu YM, Szumlanski CL, Weinshilboum RM. (1996) Human catechol-O-methyltransferase pharmacogenetics: description of a functional polymorphism and its potential application to neuropsychiatric disorders. Pharmacogenetics. 6(3):243-250.

Ladd, S. L., Gabrieli, J. D. (2015). Trait and state anxiety reduce the mere exposure effect. Frontiers in psychology, 6, 701.

Laor, N., Wolmer, L., Alon, M., Siev, J., Samuel, E., & Toren, P. (2006). Risk and protective factors mediating psychological symptoms and ideological commitment of adolescents facing continuous terrorism. The Journal of nervous and mental disease, 194(4), 279-286.

Lazarus, R.S. (1991). Emotion and adaptation. London: Oxford University Press.

Leal, P.C., Goes, T.C., da Silva, L.C.F., Teixeira-Silva, F. (2017). Ansiedade traço versus ansiedade estado em diferentes situações de ameaça. Trends in Psychiatry and Psychotherapy. 39(3):147–157.

Lee, S., Lee, M.T.Y., Kwok, K. (2005). A community-based telephone survey of social anxiety disorder in Hong Kong. Journal of Affective Disorders. 88(2):183–186.

Lee, J. K., Choi, H. G., Kim, J. Y., Nam, J., Kang, H. T., Koh, S. B., Oh, S. S. (2016). Self-resilience as a protective factor against development of post-traumatic stress disorder symptoms in police officers. Annals of Occupational and Environmental Medicine, 28(1),

Leoschut, L. (2009). Running Nowhere Fast: Results of the 2008 National Youth Lifestyle Study.

Levenson, R. W. (2014). The autonomic nervous system and emotion.Emotion Review,6(2), 100–112.

Leyfer, O.T., Ruberg, J.L., Woodruff-Borden, J. (2006). Examination of the utility of the Beck Anxiety Inventory and its factors as a screener for anxiety disorders. Journal of Anxiety Disorders. 20(4):444–458.

Liebenberg, L., Ungar, M., Van de Vijver, F. (2012). Validation of the child and youth resilience measure-28 (CYRM-28) among Canadian youth.Research on Social Work Practice.

Liehr, J. G., Roy, D. (1990). Free radical generation by redox cycling of estrogens. Free radical biology & medicine, 8(4), 415–423.

Lochner, C., du Toit, P.L., Zungu-Dirwayi, N. (2002) Childhood trauma in obsessive-compulsive disorder, trichotillomania, and controls.Depress Anxiety. 15(2):66-68.

Lochner, C., Kinnear, C.J., Hemmings, S.M.J., Seller, C., Niehaus, D.J.H., Knowles, J.A., Daniels, W., Moolman-Smook, J.C. (2005). Hoarding in obsessive-compulsive disorder: Clinical and genetic correlates. Journal of Clinical Psychiatry. 66(9):1155–1160.

Lotta, T., Vidgren, J., Tilgmann, C., Ulmanen, I., Melén, K., Julkunen, I., Taskinen, J. (1995). Kinetics of Human Soluble and Membrane-Bound Catechol O-Methyltransferase: A Revised Mechanism and Description of the Thermolabile Variant of the Enzyme. Biochemistry. 34(13):4202–4210.

Lukasik, K. M., Waris, O., Soveri, A., Lehtonen, M., Laine, M. (2019). The Relationship of Anxiety and Stress With Working Memory Performance in a Large Non-depressed Sample.Frontiers in psychology, 10.4.

Luthar, S.S., Sawyer, J.A., Brown, P.J. (2006). Conceptual issues in studies of resilience: Past, present, and future research. In Annals of the New York Academy of Sciences. V. 1094. Blackwell Publishing Inc. 105–115.

Lyons, R. F., Mickelson, K. D., Sullivan, M. J. L.,Coyne, J. C. (1998). Coping as a communal process. Journal of Social and Personal Relationships, 15(5), 579– 605

MacDonald, K., Thomas, M.L., Sciolla, A.F., Schneider, B., Pappas, K., Bleijenberg, G., Bohus, M., Bekh, B., Carpenter, L., Carr, A., Dannlowski, U., Dorahy, M., Fahlke, C., Finzi-Dottan, R., Karu, T., Gerdner, A., Glaesmer, H., Grabe, H.J., Heins, M., Kenny, D.T., Kim, D., Knoop, H., Lobbestael, J., Lochner, C., Lauritzen, G., Ravndal, E., Riggs, S., Sar, V., Schäfer, I., Schlosser, N., Schwandt, M.L., Stein, M.B., Subic-Wrana, C., Vogel, M., Wingenfeld, K. (2016). Minimization of Childhood Maltreatment Is Common and Consequential: Results from a Large, Multinational Sample Using the Childhood Trauma Questionnaire.PLOS ONE, 11(1), p.e0146058.

Madewell, AN; Ponce-Garcia, E; Bruno-Casteñeda, B; Struck-Downen, S; Taylor, HD. (2019) An abbreviation of the scale of protective factors: Resilience in a medical trauma sample Current Psychology: Research and Reviews; New York. 1-11

Manyema, M.,Richter, L.M. (2019). Adverse childhood experiences: prevalence and associated factors among South African young adults. Heliyon. 5(12)

Marx, M., Young, S.Y., Harvey, J., Rosenstein, D., Seedat, S. (2017). An Examination of Differences in Psychological Resilience between Social Anxiety Disorder and Posttraumatic Stress Disorder in the Context of Early Childhood Trauma.Frontiers in Psychology, 8.

Masten, A. S., Coatsworth, J. (1998). The development of competence in favorable and unfavourable environments: Lessons from research on successful children. American Psychologist, 53(2), 205–220.

Maul, S, Giegling, I, Fabbri, C, Corponi, F, Serretti, A, Rujescu, D. (2020) Genetics of resilience: Implications from genome-wide association studies and candidate genes of the stress response system in posttraumatic stress disorder and depression. Am J Med Genet Part B. 183: 77– 94.

McGrath, M., Kawachi, I., Ascherio, A., Colditz, G.A., Hunter, D.J., Vivo, I.D. (2004). Association between cathecol-O-methyltyranferase and phobic anxiety. American Journal of Psychiatry, 161(9), pp.1703–1705.

McLean, C.P., Asnaani, A., Litz, B.T., Hofmann, S.G. (2011). Gender differences in anxiety disorders: Prevalence, course of illness, comorbidity and burden of illness. Journal of Psychiatric Research, 45(8), pp.1027–1035.

McLaughlin, K.A., Colich, N.L., Rodman, A.M., Weissman, D.G. (2020). Mechanisms linking childhood trauma exposure and psychopathology: a transdiagnostic model of risk and resilience.BMC Medicine.18(1), pp.1–11.

McLaughlin KA, Hatzenbuehler ML, Mennin DS, Nolen-Hoeksema S. (2011) Emotion dysregulation and adolescent psychopathology: A prospective study. Behavior Research and Therapy.49:544–554.

Meichenbaum D., Cameron R (1989) Stress inoculation training. In: Meichenbaum D., Jaremko M. Stress Reduction and Prevention. New York, NY, USA: Springer

Melo-Felippe FB, de Salles Andrade JB, Giori IG, Vieira-Fonseca T, Fontenelle LF, Kohlrausch FB. (2016) Catechol-O-Methyltransferase Gene Polymorphisms in Specific Obsessive-Compulsive Disorder Patients' Subgroups. J Mol Neurosci.;58(1):129-136.

Meyer-Lindenberg, A., Nichols, T., Callicott, J.H., Ding, J., Kolachana, B., Buckholtz, J., Mattay, V.S., Egan, M., Weinberger, D.R. (2006). Impact of complex genetic variation in *COMT* on human brain function. Molecular Psychiatry, 11(9), pp.867–877, 797.

Milad MR, Wright CI, Orr SP, Pitman RK, Quirk GJ, Rauch SL. (2007) Recall of fear extinction in humans activates the ventromedial prefrontal cortex and hippocampus in concert. Biol Psychiatry. 62(5):446–54.

Miller, S. A., Dykes, D. D., Polesky, H. F. (1988). A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic acids research, 16(3), 1215

Molero, P., Ortuño, F., Zalacain, M., Patiño-García, A. (2007). Clinical involvement of catechol-O -methyltransferase polymorphisms in schizophrenia spectrum disorders: influence on the severity of psychotic symptoms and on the response to neuroleptic treatment. The Pharmacogenomics Journal, 7(6), pp.418–426.

Monroe, S. M., Slavich, G. M., Torres, L. D., Gotlib, I. H. (2007). Major life events and major chronic difficulties are differentially associated with history of major depressive episodes. Journal of Abnormal Psychology, 116(1), 116–124

Morin, A. (2011). Self-Awareness Part 1: Definition, Measures, Effects, Functions, and Antecedents. Social and Personality Psychology Compass, 5(10), pp.807–823.

Morris-Rosendahl D. J. (2002). Are there anxious genes? Dialogues in clinical neuroscience, 4(3), 251–260.

Muntingh, A.D., van der Feltz-Cornelis, C.M., van Marwijk, H.W., Spinhoven, P., Penninx, B.W. and van Balkom, A.J. (2011). Is the beck anxiety inventory a good tool to assess the severity of anxiety? A primary care study in The Netherlands study of depression and anxiety (NESDA). BMC Family Practice.

Nackley, A.G., Shabalina, S.A., Tchivileva, I.E., Satterfield, K., Korchynskyi, O., Makarov, S.S., Maixner, W., Diatchenko, L. (2006). Human Catechol-O-Methyltransferase Haplotypes Modulate Protein Expression by Altering mRNA Secondary Structure. Science, 314(5807), pp.1930–1933.

Niehaus DJ, Kinnear CJ, Corfield VA, du Toit PL, van Kradenburg J, Moolman-Smook JC (2001) Association between a catechol-omethyltransferase polymorphism and obsessive-compulsive disorder in the Afrikaner population. J Affect Disord. 65:61-5.

National Institute of Mental Health. (1998) Genetics and mental disorders: Report of the National Institute of Mental Health's Genetics Workgroup.

Nolen-Hoeksema, S., Girgus, J.S. (1994). The emergence of gender differences in depression during adolescence.Psychological Bulletin, 115(3), pp.424–443.

Norman, R.E., Byambaa, M., De, R., Butchart, A., Scott, J., Vos, T. (2012). The Long-Term Health Consequences of Child Physical Abuse, Emotional Abuse, and Neglect: A Systematic Review and Meta-Analysis. PLoS Medicine, 9(11), p.e1001349.

Norrholm, S. D., Ressler, K. J. (2009). Genetics of anxiety and trauma-related disorders. Neuroscience, 164(1), 272–287.

Nota, L., Soresia, S., Zimmerman, B. J. (2004). Self-regulation and academic achievement and resilience: A longitudinal study. International Journal of Educational Research, 41(3), 198–215.

Ohara, K., Nagai, M., Suzuki, Y., Ochiai, M. (1998). No association between anxiety disorders and catechol-O-methyltransferase polymorphism.Psychiatry Research, 80(2), pp.145–148.

Olsson, C.A., Anney, R.J.L., Lotfi-Miri, M., Byrnes, G.B., Williamson, R., Patton, G.C. (2005). Association between the COMT Val158Met polymorphism and propensity to anxiety in an Australian population-based longitudinal study of adolescent health.Psychiatric Genetics, 15(2), pp.109–115.

Ozturk, A.R., Can, T. (2017). A multiplex primer design algorithm for target amplification of continuous genomic regions. BMC Bioinformatics, 18(1).

Palais, R.A., Liew, M.A., Wittwer, C.T. (2005). Quantitative heteroduplex analysis for single nucleotide polymorphism genotyping. Anal. Biochem. 346: 167-175

Patton, G.C., Coffey, C., Romaniuk, H., Mackinnon, A., Carlin, J.B., Degenhardt, L., Olsson, C.A., Moran, P. (2014). The prognosis of common mental disorders in adolescents: a 14-year prospective cohort study. The Lancet, 383(9926), pp.1404–1411.

Pawelczyk K. (2012) 'Believe in zero' campaign aims to end violence against children in South Africa.

Peng H, Zhu Y, Strachan E, Fowler E, Bacus, T., Roy-Byrne, P., Goldberg, J., Vaccarine, V., Zhao, J. (2018) Childhood Trauma, DNA Methylation of Stress-Related Genes, and Depression: Findings From Two Monozygotic Twin Studies. Psychosomatic Medicine. 80(7):599-608.

Pennebaker, J.W., Susman, J.R. (1988). Disclosure of traumas and psychosomatic processes.Social Science & Medicine, 26(3), pp.327–332.

Perroud, N., Jaussent, I., Guillaume, S., Bellivier, F., Baud, P., Jollant, F., Leboyer, M., Lewis, C. M., Malafosse, A., Courtet, P. (2010). COMT but not serotonin-related genes modulates the influence of childhood abuse on anger traits. Genes, brain, and behavior, 9(2), 193–202.

Petersen, A.C., Joseph, J., Feit, M. (2014) Committee on Child Maltreatment Research, Policy, and Practice for the Next Decade: Phase II, Board on Children, Youth, and Families, Committee on Law and Justice, Institute of Medicine and National Research Council. Consequences of Child Abuse and Neglect.

Philippe, F., Laventure, S., Beaulieu-Pelletier, G., Lecours, S., Lekes, N (2011). Ego-Resiliency as a Mediator between Childhood Trauma and Psychological Symptoms. Journal of Social and Clinical Psychology. 30. 583-598.

Ponce-Garcia, E., Madewell, A.N., Kennison, S.M. (2015). The Development of the Scale of Protective Factors: Resilience in a Violent Trauma Sample. Violence and Victims, 30(5), pp.735–755.

Purves, K.L., Coleman, J.R.I., Meier, S.M., Rayner, C., Davis, K.A.S., Cheesman, R., Bækvad-Hansen, M., Børglum, A.D., Wan Cho, S., Jürgen Deckert, J., Gaspar, H.A., Bybjerg-

Grauholm, J., Hettema, J.M., Hotopf, M., Hougaard, D., Hübel, C., Kan, C., McIntosh, A.M., Mors, O., Bo Mortensen, P., Nordentoft, M., Werge, T., Nicodemus, K.K., Mattheisen, M., Breen, G., Eley, T.C. (2019). A major role for common genetic variation in anxiety disorders. Molecular Psychiatry.

Qayyum, A., Zai, C.C., Hirata, Y., Tiwari, A.K., Cheema, S., Nowrouzi, B., Beitchman, J.H. and Kennedy, J.L. (2015). The Role of the Catechol-o-methyltransferase (COMT) Gene Val158Met in Aggressive Behavior, A Review of Genetic Studies.Current Neuropharmacology, 13(6), pp.802–814.

Quintáns, B., Alvarez-Iglesias, V., Salas, A., Phillips, C., Lareu, M. V., Carracedo, A. (2004). Typing of mitochondrial DNA coding region SNPs of forensic and anthropological interest using SNaPshot minisequencing. Forensic science international, 140(2-3), 251–257.

Ungar, M., Liebenberg, L. (2013). A Measure of Resilience with Contextual Sensitivity—The CYRM-28: Exploring the Tension Between Homogeneity and Heterogeneity in Resilience Theory and Research. In S. Prince-Embury & D. H. Saklofske (Eds.), Resilience in children, adolescents and adults: Translating research into practice (pp. 245-255). New York, NY: Springer.

Rafalski A. (2002) Applications of single nucleotide polymorphisms in crop genetics. Curr Opin Plant Biol.5(2):94-100

Rapee, R.M. (2002). The development and modification of temperamental risk for anxiety disorders: prevention of a lifetime of anxiety? Biological Psychiatry, 52(10), pp.947–957.

Reich, J. W., Zautra, A. J., Hall, J. (2010). Handbook of adult resilience. New York, NY: Guilford Press.

Reivich, K. and Shatté, A. (2002) The Resilience Factor: 7 Essential Skills for Overcoming Life's Inevitable Obstacles. Broadway Books.

Ririe, K.M., Rasmussen, R.P., Wittwer, C.T. (1997). Product differentiation by analysis of DNA melting curves during the polymerase chain reaction. Analytical biochemistry, 245(2), pp.154–60.

Rosengren E. (1960). On the Role of Monoamine Oxidase for the Inactivation of Dopamine in Brain. Acta Physiologica Scandinavica, 49(4), pp.370–375.

Roten, L.T., Fenstad, M.H., Forsmo, S., Johnson, M.P., Moses, E.K., Austgulen, R., Skorpen, F. (2011). A low *COMT* activity haplotype is associated with recurrent preeclampsia in a

Norwegian population cohort (HUNT2). Molecular Human Reproduction. 17(7), pp.439–446.

Rothe, J.A. (1992). Membrane-bound catechol-O-methyltransferase: a reevaluation of its role in the O-methylation of the catecholamine neurotransmitters.Reviews of Physiology, Biochemistry and Pharmacology.120, pp.1–29.

Rothe, C., Koszycki, D., Bradwejn, J., King, N., Deluca, V., Tharmalingam, S., Macciardi, F., Deckert, J., Kennedy, J.L. (2006). Association of the Val158Met Catechol O - Methyltransferase Genetic Polymorphism with Panic Disorder.Neuropsychopharmacology. 31(10), pp.2237–2242.

Roussos, P., Giakoumaki, S.G., Pavlakis, S. and Bitsios, P. (2008). Planning, decision-making and the COMT rs4818 polymorphism in healthy males. Neuropsychologia, 46(2), pp.757–763.

Rutter, M. (1985). Resilience in the face of adversity. Protective factors and resistance to psychiatric disorder.The British Journal of Psychiatry, 147(6), pp.598–611.

Rutter, M. (1987). Psychosocial resilience and protective mechanisms.American Journal of Orthopsychiatry, 57(3), pp.316–331.

Rutter M. (2007). Resilience, competence, and coping. Child Abuse Negl. 31, 205–209.

Saal, W. L., Kagee, A., Bantjes, J. (2019). Evaluation of the Beck Anxiety Inventory in predicting generalised anxiety disorder among individuals seeking HIV testing in the Western Cape Province, South Africa. SAJP: The journal of the Society of Psychiatrists of South Africa, 25, 1336.

Salisu, I. and Hashim, N. (2017). A Critical Review of Scales Used in Resilience Research.IOSR Journal of Business and Management, 19(04), pp.23–33.

Samochowiec, J., Hajduk, A., Samochowiec, A., Horodnicki, J., Stępień, G., Grzywacz, A. and Kucharska-Mazur, J. (2004). Association studies of MAO-A, COMT, and 5-HTT genes polymorphisms in patients with anxiety disorders of the phobic spectrum.Psychiatry Research, 128(1), pp.21–26

Sanchez, J.J., Phillips, C., Børsting, C., Balogh, K., Bogus, M., Fondevila, M., Harrison, C.D., Musgrave-Brown, E., Salas, A., Syndercombe-Court, D., Schneider, P.M., Carracedo, A. and Morling, N. (2006). A multiplex assay with 52 single nucleotide polymorphisms for human identification.Electrophoresis, 27(9), pp.1713–1724

Savitz, J.B., van der Merwe, L., Newman, T.K., Solms, M., Stein, D.J., Ramesar, R.S. (2007). The relationship between childhood abuse and dissociation. Is it influenced by catechol-O-methyltransferase (*COMT*) activity? The International Journal of Neuropsychopharmacology, 11(02).

Scanlon, P.D., Raymond, F.A., Weinshilboum, R.M. (1979). Catechol-O-methyltransferase: thermolabile enzyme in erythrocytes of subjects homozygous for allele for low activity.Science, 203(4375), pp.63–65.

Schwarzer, R., Bäßler,J., Kwiatek,P., Schröder, K., Zhang, J.X. (1997). The assessment of optimistic self – beliefs: Comparison of the German, Spanish, and Chinese versions of the general self-efficacy scale. Applied Psychology: An International Review, 46(1), 69 -88.

Seedat, S., Stein, D.J., Jackson, P.B., Heeringa, S.G., Williams, D.R. and Myer, L. (2009). Life stress and mental disorders in the South African Stress and Health study.South African medical journal = Suid-Afrikaanse tydskrif vir geneeskunde, 99(5 Pt 2), pp.375–382.

Shewchuk, R.M., Richards, J.S., Elliott, T.R. (1998). Dynamic processes in health outcomes among caregivers of patients with spinal cord injuries.Health Psychology: Official Journal of the Division of Health Psychology, American Psychological Association, 17(2), pp.125–129.

Shifman, S., Bronstein, M., Sternfeld, M., Pisanté-Shalom, A., Lev-Lehman, E., Weizman, A., Reznik, I., Spivak, B., Grisaru, N., Karp, L., Schiffer, R., Kotler, M., Strous, R.D., Swartz-Vanetik, M., Knobler, H.Y., Shinar, E., Beckmann, J.S., Yakir, B., Risch, N., Zak, N.B., Darvasi, A. (2002). A Highly Significant Association between a COMT Haplotype and Schizophrenia. The American Journal of Human Genetics, 71(6), pp.1296–1302.

Simeon, D., Yehuda, R., Cunill, R., Knutelska, M., Putnam, F.W. and Smith, L.M. (2007). Factors associated with resilience in healthy adults.Psychoneuroendocrinology, 32(8–10), pp.1149–1152.

Simon, N.M., Herlands, N.N., Marks, E.H., Mancini, C., Letamendi, A., Li, Z., Pollack, M.H., Van Ameringen, M., Stein, M.B. (2009). Childhood maltreatment linked to greater symptom severity and poorer quality of life and function in social anxiety disorder. Depression and Anxiety, 26(11), pp.1027–1032.

Sjoerds Z, Van Den Brink W, Beekman AT, Penninx BW, Veltman DJ. (2014) Response inhibition in alcohol-dependent patients and patients with depression/anxiety: a functional magnetic resonance imaging study. Psychol Med. 44(8):1713–25.

Smith, B.W., Dalen, J., Wiggins, K., Tooley, E., Christopher, P., Bernard, J. (2008). The brief resilience scale: Assessing the ability to bounce back.International Journal of Behavioral Medicine, 15(3), pp.194–200.

Smolka, M.N. (2005). Catechol-O-Methyltransferase val158met Genotype Affects Processing of Emotional Stimuli in the Amygdala and Prefrontal Cortex..Journal of Neuroscience, 25(4), pp.836–842.

Sneed, J.R., Johnson, J.G., Cohen, P., Gilligan, C., Chen, H., Crawford, T.N., Kasen, S. (2006). Gender differences in the age-changing relationship between instrumentality and family contact in emerging adulthood. Developmental Psychology, 42(5), 787-797.

Sonn, C.C., Fisher, A.T. (1998). Sense of community: Community resilient responses to oppression and change.Journal of Community Psychology. 26(5), pp.457–472.

South African Depression & Anxiety Group". Sadag.org. Retrieved 2019-12-03.

Spielberger, C. D. (1989).State-Trait Anxiety Inventory: Bibliography (2nd Ed.). Palo Alto, CA: Consulting Psychologists Press.South African Depression and Anxiety Group.

Spielberger, C. D., Gorsuch, R. L., Lushene, R., Vagg, P. R., Jacobs, G.(1983) A.Manual for the state-trait anxiety inventory

Southwick, S.M., Charney, D.S. (2012). The Science of Resilience: Implications for the Prevention and Treatment of Depression.Science, 338(6103), pp.79–82.

Spielman, R.S., Weinshilboum, R.M., Opitz, J.M. (1981). Genetics of red cell *COMT* activity: Analysis of thermal stability and family data.American Journal of Medical Genetics, 10(3), pp.279–290.

Stanley MA, Novy DM, Bourland SL, Beck JG, Averill PM. (2001). Assessing older adults with generalized anxiety: a replication and extension. Beh Res and Ther 39: 221–225.

Stansfeld, S.A., Rothon, C., Das-Munshi, J., Mathews, C., Adams, A., Clark, C., Lund, C. (2017). Exposure to violence and mental health of adolescents: South African Health and Well-being Study.BJPsych Open, 3(5), pp.257–264.

Statistics South Africa (2019). Five facts about poverty in South Africa. Statistics South Africa.

Steckler, T., Salvadore, G. (2013). Chapter 7 - Neuroimaging as a Translational Tool in Animal and Human Models of Schizophrenia. ScienceDirect.

Stein, M.B., Fallin, M.D., Schork, N.J., Gelernter, J. (2005). COMT Polymorphisms and Anxiety-Related Personality Traits.Neuropsychopharmacology, 30(11), pp.2092–2102.

Stein MB, Jang KL, Taylor S, Vernon PA, Livesley WJ. (2002) Genetic and environmental influences on trauma exposure and posttraumatic stress disorder symptoms: a twin study. The American journal of psychiatry.159:1675–1681

Stewart, D.W., Mandrusiak, M. (2007). Social Phobia in College Students.Journal of College Student Psychotherapy, 22(2), pp.65–76.

Stevens, J. E. (2012). The adverse childhood experiences study – The largest, most important public health study you never heard of – Began in an obesity clinic.

Stoltenborgh, M., Bakermans-Kranenburg, M.J., Alink, L.R.A., van IJzendoorn, M.H. (2014). The Prevalence of Child Maltreatment across the Globe: Review of a Series of Meta-Analyses. Child Abuse Review, 24(1), pp.37–50.

Suliman, S., Kaminer, D., Seedat, S., Stein, D. (2005). Assessing post-traumatic stress disorder in South African adolescents: Using the child and adolescent trauma survey (CATS) as a screening tool.Annals of General Psychiatry, 4(1), p.2.

Sun, J., Stewart, D. (2007). Age and Gender Effects on Resilience in Children and Adolescents.International Journal of Mental Health Promotion, 9(4), pp.16–25.

Teicher, M.H., Samson, J.A., Anderson, C.M., Ohashi, K. (2016). The effects of childhood maltreatment on brain structure, function and connectivity. Nature Reviews Neuroscience, 17(10), pp.652–666.

Tenhunen, J., Salminen, M., Lundström, K., Kiviluoto, T., Savolainen, R., Ulmanen, I. (1994). Genomic organization of the human catechol O-methyltransferase gene and its expression from two distinct promoters.European Journal of Biochemistry, 223(3), pp.1049–1059.

Theron, Linda C., Theron, Adam M.C. (2010). A critical review of studies of South African youth resilience, 1990-2008. South African Journal of Science, 106(7-8), 1-8

Thombs, B.D., Bernstein, D.P., Lobbestael, J., Arntz, A. (2009). A validation study of the Dutch Childhood Trauma Questionnaire-Short Form: Factor structure, reliability, and known-groups validity.Child Abuse & Neglect, 33(8), pp.518–523.

Tolin, D.F., Foa, E.B. (2006). Sex differences in trauma and posttraumatic stress disorder: A quantitative review of 25 years of research.Psychological Bulletin, 132(6), pp.959–992.

Tomasi, J., Zack, M., Kennedy, J.L. (2019). Outside the typical anxiety disorder definition: Characterizing the role of impulsivity in comorbid substance use disorder. Personalized Medicine in Psychiatry, 15–16, pp.13–21.

Tottenham N, Hare TA, Millner A, Gilhooly T, Zevin JD, Casey BJ. (2011) Elevated amygdala response to faces following early deprivation. Dev Sci.14:190–204.

Tsuang, M. T., Bar, J. L., Stone, W. S., Faraone, S. V. (2004). Gene-environment interactions in mental disorders. World psychiatry: official journal of the World Psychiatric Association (WPA), 3(2), 73–83.

Vallone, P.M., Just, R.S., Coble, M.D., Butler, J.M, Parsons, T.J. (2004). A multiplex allele-specific primer extension assay for forensically informative SNPs distributed throughout the mitochondrial genome.International Journal of Legal Medicine.118 (3), pp.147–157.

Van Devender, A. (2014). The Impact of Childhood Experiences on Intrapersonal and Interpersonal Functioning: Does the Past Dictate the Future? USC Aiken Psychology Theses.

van Harmelen, A. L., van Tol, M. J., Demenescu, L. R., van der Wee, N. J., Veltman, D. J., Aleman, A., van Buchem, M. A., Spinhoven, P., Penninx, B. W., Elzinga, B. M. (2013). Enhanced amygdala reactivity to emotional faces in adults reporting childhood emotional maltreatment. Social cognitive and affective neuroscience, 8(4), 362–369.

van der Kolk B. (2005) Developmental trauma disorder: Toward a rational diagnosis for children with complex trauma histories. Psychiatric Annals.35:401–408.

van Rooij, S. J., Stevens, J. S., Ely, T. D., Fani, N., Smith, A. K., Kerley, K. A., Lori, A., Ressler, K. J., & Jovanovic, T. (2016). Childhood Trauma and *COMT* Genotype Interact to Increase Hippocampal Activation in Resilient Individuals. Frontiers in psychiatry, 7, 156.

Verbruggen, F., Logan, G. D. (2008). Response inhibition in the stop-signal paradigm. Trends in cognitive sciences, 12(11), 418–424.

Villano, C.L., Cleland, C., Rosenblum, A., Fong, C., Nuttbrock, L., Marthol, M., Wallace, J. (2004). Psychometric Utility of the Childhood Trauma Questionnaire with Female Street-Based Sex Workers. Journal of Trauma & Dissociation, 5(3), pp.33–41.

Volavka, J., Bilder, R., Noland, K. (2004), Catecholamines and Aggression: The Role of COMT and MAO Polymorphisms. Annals of the New York Academy of Sciences, 1036(1): pp393-398.

Walker, E.C., Holman, T.B., Busby, D.M. (2009). Childhood Sexual Abuse, Other Childhood Factors, and Pathways to Survivors' Adult Relationship Quality. Journal of Family Violence, 24(6), pp.397–406.

Walsh, F. (2006). Strengthening family resilience. New York: Guilford Publications

Wang MC, Haertel GD, Wahlberg HJ. (1994) Educational resilience in inner cities. In: Wang MC, Gordon EW, editors. Educational resilience in inner-city America: Challenges and prospects. Erlbaum; Hillsdale, NJ: 1994. pp. 45–72

Ward, C., Artz, L., Berg, J., Boonzaier, F., Crawford-Browne, S., Dawes, A., Foster, D., Matzopoulos, R., Nicol, A., Seekings, J., van As, A., van der Spuy, E. (2012). Violence, violence prevention, and safety: A research agenda for South Africa.South African Medical Journal, 102(4), 215-218

Weinshilboum, R.M., Otterness, D.M., Szumlanski, C.L. (1999). Methylation pharmacogenetics: catechol O-methyltransferase, thiopurine methyltransferase, and histamine N-methyltransferase. Annual Review of Pharmacology and Toxicology, 39, pp.19–52.

Werner, E. (1997). Vulnerable but invincible: high-risk children from birth to adulthood. Acta Paediatrica, 86(S422), pp.103–105.

Werner EE, Smith RS, (1992) Overcoming the odds: High risk children from birth to adulthood. Cornell University Press; Ithaca, NY:

Wesselhoeft, R., Pedersen, C.B., Mortensen, P.B., Mors, O., Bilenberg, N. (2014). Gender–age interaction in incidence rates of childhood emotional disorders. Psychological Medicine, 45(4), pp.829–839.

Wetherell, J.L., Gatz, M. (2005). The Beck Anxiety Inventory in Older Adults With Generalized Anxiety Disorder. Journal of Psychopathology and Behavioral Assessment, 27(1), pp.17–24.

Wheaton, B. (1999). Social stress. In C. S. Aneshensel & J. C. Phelan (Eds.), Handbook of sociology and social research. Handbook of sociology of mental health. p. 277–300.

Windle, G., Bennett, K.M. and Noyes, J. (2011). A methodological review of resilience measurement scales. Health and Quality of Life Outcomes, 9(1), p.8.

Wittchen, H.-U., Stein, M.B. Kessler, R.C. (1999). Social fears and social phobia in a community sample of adolescents and young adults: prevalence, risk factors and co-morbidity. Psychological Medicine, 29(2), pp.309–323.

Wittwer, C.T., Reed, G.H., Gundry, C.N., Vandersteen, J.G., Pryor, R.J. (2003). High-resolution genotyping by amplicon melting analysis using LCGreen. Clinical Chemistry, 49(6 Pt 1), pp.853–860.

Wolkow, E, K., Ferguson, B, H. (2001). Community Factors in the Development of Resiliency: Considerations and Future In Directions in Community Mental Health Journal Vol.37 (6) Human Science Press Inc.

Wong, M. (2008). Perceptions of Parental Involvement and Autonomy Support: Their Relations with Self-Regulation, Academic Performance, Substance Use and Resilience among Adolescents. North American Journal of Psychology, 10(3), pp.497–518.

Wray, N.R., James, M.R., Dumenil, T., Handoko, H.Y., Lind, P.A., Montgomery, G.W., Martin, N.G. (2008). Association study of candidate variants of *COMT* with neuroticism, anxiety and depression. American Journal of Medical Genetics Part B: Neuropsychiatric Genetics, 147B(7), pp.1314–1318.

Wright, A., Charlesworth, B., Rudan, I., Carothers, A., Campbell, H. (2003). A polygenic basis for late-onset disease. Trends in genetics: TIG, 19(2), pp.97–106.

Wu, G., Feder, A., Cohen, H., Kim, J. J., Calderon, S., Charney, D. S., Mathé, A. A. (2013). Understanding resilience. Frontiers in behavioral neuroscience, 7, 10.

Wu H, Dong F, Wang Y, Xiao Q, Yang Q, Zhao J, Quinn TJ, Chen SD, Liu J. (2014) Catechol-O-methyltransferase Val158Met polymorphism: modulation of wearing-off susceptibility in a Chinese cohort of Parkinson's disease. Parkinsonism Relat Disord. 20:1094–6.

You, F.M., Huo, N., Gu, Y.Q., Luo, M.-C., Ma, Y., Hane, D., Lazo, G.R., Dvorak, J., Anderson, O.D. (2008). BatchPrimer3: a high throughput web application for PCR and sequencing primer design. BMC bioinformatics, 9, p.253.

Zandbelt BB, Van Buuren M, Kahn RS, Vink M. (2011) Reduced proactive inhibition in schizophrenia is related to corticostriatal dysfunction and poor working memory.Biol Psychiatry. 70(12):1151–8.

Zhang, Y., Feng, S., Nie, K., Zhao, X., Gan, R., Wang, L., Zhao, J., Tang, H., Gao, L., Zhu, R., Wang, L., Zhang, Y. (2016). Catechol-O-methyltransferase Val158Met polymorphism

influences prefrontal executive function in early Parkinson's disease. Journal of the Neurological Sciences, 369, pp.347–353.

Zhang, K., Qu, S., Chang, S., Li, G., Cao, C., Fang, K., Olff, M., Wang, L., Wang, J. (2017). An overview of posttraumatic stress disorder genetic studies by analyzing and integrating genetic data into genetic database PTSD gene. Neuroscience and Biobehavioral Reviews, 83, pp.647–656.

Zhang, K., Wang, L., Cao, C., Li, G., Fang, R., Liu, P., Luo, S., Zhang, X., Liberzon, I. (2018). A DRD2/ANNK1–COMT Interaction, Consisting of Functional Variants, Confers Risk of Post-traumatic Stress Disorder in Traumatized Chinese. Frontiers in Psychiatry, 9.

Züchner, S., Roberts, S.T., Speer, M.C. and Beckham, J.C. (2007). Update on psychiatric genetics. Genetics in Medicine, 9(6), pp.332–340.

Appendix I

UNIVERSITY OF THE WESTERN CAPE

Private Bag X 17, Bellville 7535, South Africa

Tel: +27 21 959 2065/2216, Fax: +27 21 9591325

E-mail: **sdavison@uwc.ac.za, 3135264@myuwc.ac.za; 3211728@myuwc.ac.za**

CONSENT FORM

Project Title: A neuro-endophenotypic approach to understanding the genetics underlying anxiety and reward motivation behaviour and the association of resilience in South Africa

- The study has been described to me in a language that I understand and I freely and voluntarily agree to participate in it.
- My questions about the study have been answered.
- I understand that my identity is kept confidential and that I may withdraw from the study without giving a reason at any time and this will not negatively affect me in any way.
- The genetic material for analysis is to be obtained from the swab/saliva I am donating.
- The sample I provided will be assigned a unique identification number and stored with reference to my name and surname.
- The sample will be stored until termination of the study and will then be destroyed.

- I agree to have non-invasive electrodes placed on my scalp to measure brain wave patterns (electroencephalography).
- I agree to provide information about gender, ancestry and ethnicity.

Participant's name and surname	**Participant's signature**	**Date**

Should participants have any questions regarding this study or which to report any problems they have experienced related to the study, they should please contact the study coordinator.

Study Coordinator's Details:

Prof Sean Davison

Tel: *021 9592216*

E-mail: *SDavison@uwc.ac.za*

Address: *University of the Western Cape,*

Forensic DNA Laboratory, Private Bag X 17,

Bellville 7535, South Africa.

Researchers Details:

Sarah Jacobs

E-mail: *3135264@myuwc.ac.za*

Appendix II

Sample number

UNIVERSITY of the WESTERN CAPE

University of the Western Cape

Genetic sample collection and screening questionnaire

Please answer all sections. All information collected for this study will remain confidential.

First Name

Surname

Sex (circle)

Date of birth: DD/MM/YYYY

Age:

Please circle if applicable

Are you South African?	YES	NO
Is your biological mother South African?	YES	NO
Is your biological father South African?	YES	NO
Are your mothers parents South African?	YES	NO
Are your fathers parents South African?	YES	NO

Which country were you born in?

How long have you been living in South Africa for?

Please complete the table below as best you can:

	Mother's mother	Mother	Own	Father's	Father's father
Population Group					
Home Language					
Date of Birth					
Place of Birth					

Email Address

Contact Number

What is your preferred method of contact?

EMAIL	PHONECALL	SMS

Appendix III

Table 1.1 SNP flanking primer report generated for Rs2075507 (BatchPrimer 3)

	Orientation	Start	Len	Tm	GC%	Any compl	3' compl	Primer Seq	Product Size	Seq Size	Included Size	Pair_any	Pair_3'
1	FORWARD	56	20	59.97	45.00	6.00	1.00	AATTTGGCTATTGCCGTGTC	117	201	201	4.00	1.00
	REVERSE	172	20	60.15	45.00	5.00	0.00	CAAAGGGCATTTATCATGGG					
2	FORWARD	55	20	59.97	45.00	6.00	2.00	GAATTTGGCTATTGCCGTGT	118	201	201	4.00	1.00
	REVERSE	172	20	60.15	45.00	5.00	0.00	CAAAGGGCATTTATCATGGG					
3	FORWARD	55	20	59.97	45.00	6.00	2.00	GAATTTGGCTATTGCCGTGT	100	201	201	5.00	2.00
	REVERSE	154	20	59.79	50.00	5.00	2.00	GGGTTCAGAATCACGGATGT					
4	FORWARD	62	20	60.29	55.00	4.00	2.00	GCTATTGCCGTGTCTGGACT	111	201	201	4.00	0.00
	REVERSE	172	20	60.15	45.00	5.00	0.00	CAAAGGGCATTTATCATGGG					
5	FORWARD	56	20	59.97	45.00	6.00	1.00	AATTTGGCTATTGCCGTGTC	114	201	201	4.00	0.00
	REVERSE	169	20	60.40	45.00	4.00	0.00	AGGGCATTTATCATGGGGTT					

Table 1.2 SNP flanking primer report generated for Rs737865 (BatchPrimer 3)

	Orientation	Start	Len	Tm	GC%	Any compl	3' compl	Primer Seq	Product Size	Seq Size	Included Size	Pair_any	Pair_3'
1	FORWARD	79	20	60.05	40.00	4.00	1.00	TTTTGGATTTTTCCAGCCAG	113	201	201	4.00	2.00
	REVERSE	191	20	60.62	60.00	5.00	2.00	AGTGTCTCACTGGGCTCTGC					
2	FORWARD	14	20	60.23	55.00	8.00	0.00	GGACCACGTGGGAATGTTAG	178	201	201	5.00	1.00
	REVERSE	191	20	60.62	60.00	5.00	2.00	AGTGTCTCACTGGGCTCTGC					
3	FORWARD	13	20	60.23	50.00	8.00	2.00	AGGACCACGTGGGAATGTTA	179	201	201	5.00	0.00
	REVERSE	191	20	60.62	60.00	5.00	2.00	AGTGTCTCACTGGGCTCTGC					
4	FORWARD	78	20	60.41	35.00	4.00	0.00	TTTTGGATTTTTCCAGCCA	114	201	201	4.00	1.00
	REVERSE	191	20	60.62	60.00	5.00	2.00	AGTGTCTCACTGGGCTCTGC					
5	FORWARD	34	20	60.62	55.00	5.00	3.00	AGAAAGGGGAAGTCACTCCG	158	201	201	5.00	1.00
	REVERSE	191	20	60.62	60.00	5.00	2.00	AGTGTCTCACTGGGCTCTGC					

Table 1.3 SNP flanking primer report generated for Rs6269 (BatchPrimer 3)

	Orientation	Start	Len	Tm	GC%	Any compl	3' compl	Primer Seq	Product Size	Seq Size	Included Size	Pair_any	Pair_3'
1	FORWARD	44	21	62.07	57.14	8.00	3.00	CTGACACGTCAGGCAACTGAG	130	201	201	5.00	2.00
	REVERSE	173	20	60.76	60.00	6.00	0.00	CAGTGCTCTGTGCTCCTCCT					

Table 1.4 SNP flanking primer report generated for Rs4633 (BatchPrimer 3)

	Orientation	Start	Len	Tm	GC%	Any compl	3' compl	Primer Seq	Product Size	Seq Size	Included Size	Pair_any	Pair_3'
1	FORWARD	25	20	60.80	55.00	6.00	2.00	GCTGGAACGAGTTCATCCTG	149	201	201	4.00	1.00
	REVERSE	173	21	60.21	57.14	8.00	1.00	CTTCTGCTCGCAGTAGGTGTC					

Table 1.5 SNP flanking primer report generated for Rs4818 (BatchPrimer 3)

	Orientation	Start	Len	Tm	GC%	Any compl	3' compl	Primer Seq	Product Size	Seq Size	Included Size	Pair_any	Pair_3'
1	FORWARD	40	21	61.20	61.90	5.00	3.00	GGGCCTACTGTGGCTACTCAG	146	201	201	4.00	1.00
	REVERSE	185	21	61.57	52.38	4.00	0.00	CATGCACACCTTGTCCTTCAC					

Table 1.6 SNP flanking primer report generated for Rs4680 (BatchPrimer 3)

	Orientation	Start	Len	Tm	GC%	Any compl	3' compl	Primer Seq	Product Size	Seq Size	Included Size	Pair_any	Pair_3'
1	FORWARD	35	22	61.82	50.00	6.00	0.00	CTCATCACCATCGAGATCAACC	117	201	201	4.00	2.00
	REVERSE	151	20	60.08	50.00	5.00	3.00	CCCTTTTTCCAGGTCTGACA					

Table 1.7 The genotypes for all six *COMT* SNPs for sub set of the population group,

Sample ID	rs4633		rs680		rs4818		rs6269		rs737865		rs2075507	
	Length	*Genotype*	*Length*	*Genotype*	*Length*	*Genotype*	*Length*	*Genotype*	*Length*	*Genotype*	*Length*	*Genotype*
10	-	-	36	G	38	G	43	A	47	A	50	A
22	-	-	36	G	39	C	41	G	47	A	51	A
40	-	-	36	G	38	C	41	G	47	A	51	A
60	-	-	36	G	40	G	43	A	47	A	51	A
76	-	C	37	A	41	C	44	G	49	G	51	A
78	33	C	37	A	40	C	41	G	44	A	51	A
80	34	C	37	A	-	-	45	G	44	A	51	A
83	34	C	37	A	38	C	41	G	45	A	-	-
85	34	C	38	A	41	C	44	G	49	A	52	A
90	34	C	37	G	41	C	44	G	48	A	51	A
92	34	C	37	A	41	C	43	G	48	A	51	A

Table 1.8 Primer BLAST report for rs737865

Location	Product length	Features flanking product
Homo sapiens chromosome 22, GRCh38.p13	113bp	19838 bp at 3' side: catechol O-methyltransferase isoform MB-COMT
		774 bp at 5' side: thioredoxin reductase 2, mitochondrial isoform 5 precursor
Homo sapiens chromosome 15, GRCh38.p13	1988bp	nuclear receptor ROR-alpha isoform X2, X3
Homo sapiens chromosome 6, GRCh38.p13	3858bp	translocating chain-associated membrane protein 2
		translocating chain-associated membrane protein 2 isoform X1
	1977bp	10695 bp at 5' side: tubulin beta-2B chain
		14649 bp at 3' side: proteasome assembly chaperone 4 isoform X4
Homo sapiens chromosome 3, GRCh38.p13	1079bp	81427 bp at 5' side: disks large homolog 1 isoform X14
		132195 bp at 3' side: D-beta-hydroxybutyrate dehydrogenase, mitochondrial isoform
Homo sapiens chromosome 5, GRCh38.p13	3441bp	10524 bp at 5' side: GRAM domain-containing protein 2B isoform X10
		42049 bp at 3' side: alpha-aminoadipic semialdehyde dehydrogenase isoform 2
Homo sapiens chromosome 8, GRCh38.p13	3329bp	F-box only protein 32 isoform 3, 1
Homo sapiens chromosome X, GRCh38.p13	2219bp	70907 bp at 5' side: bone morphogenetic protein 15 preproprotein
		343234 bp at 3' side: diphosphoinositol polyphosphate phosphohydrolase 3-alpha
Homo sapiens chromosome 2, GRCh38.p13	1419bp	8462 bp at 5' side: gastrokine-1 precursor
		22797 bp at 3' side: anthrax toxin receptor 1 isoform 1 precursor

Homo sapiens chromosome 1, GRCh38.p13	986bp	protein-cysteine N-palmitoyltransferase HHAT isoform 4
		protein-cysteine N-palmitoyltransferase HHAT isoform X5
Homo sapiens chromosome 7, GRCh38.p13	2703bp	dipeptidyl aminopeptidase-like protein 6 isoform 6
	956BP	dedicator of cytokinesis protein 4 isoform X4, X10
Homo sapiens chromosome 17, GRCh38.p13	2341BP	zinc phosphodiesterase ELAC protein 2 isoform X1, X7

Appendix IV

Self-report scales questionnaire (transferred and completed via Google forms)

** Indicates the section/scale title. This was not available to the participant*

Please answer all sections.

If you are unsure of a question, please raise your hand for assistance.

Student Number:	
Initials:	
Age:	
Sex (Male/Female):	
Date of birth:	
Highest level of education completed:	
Who do you live with?	
How long have you been living with these people?	
How many times have you moved homes in the past 5 years?	
Please describe who you consider to be your family?	
People are often described as belonging to a particular racial group. To which of the following group(s) do you belong? (Mark or check the one(s) that best describe(s) you.)	□ Aboriginal or Native □ South Asian (*e.g., East Indian, Pakistani, Punjabi, Sri Lankan)* □ South-East Asian (*e.g., Cambodian, Indonesian, Laotian, Vietnamese)* □ West Asian to Middle Eastern (*e.g., Armenian, Egyptian, Iranian, Lebanese)* □ Asian (*e.g., Korean, Chinese, Japanese)* □ Black (*e.g., African or Caribbean descent)* □ White or European □ Filipino □ Latin American (*e.g., Mexican, South American, Central American)* □ Other (please specify): □ Mixed Race (please list all groups that apply):
People are often described as belonging to a particular ethnic or cultural group(s). (For example, Chinese, Jamaican, German, Italian, Irish, English, East Indian, Jewish, Scottish, Portuguese, French,etc.) To which ethnic or cultural group(s) do you see yourself belonging? Please list as many groups as you want	

1. If you are a female, please indicate if you are taking any form or contraceptive (oral/injection)

YES	NO

2. In the last two years, have you been prescribed any form of chronic medication by a doctor?

YES	NO

If you circled YES above (question 2), please answer the following questions.

If you circled NO, please move onto the next section, question 3.

- If you are on chronic medication prescribed by a medical practioner, is it for a medical condition?
- Please indicate the medical condition: _____
- Please tick the appropriate boxes which apply to you:

Condition:	**Please tick if applicable:**
Diabetes	
Blood pressure	
Cholesterol	
Mental health disorder	
Other	

3. Have you lost consciousness for more than a few moments or experienced significant brain trauma (surgery/skull fracture)?

YES	NO

If you circled YES above (question 3), please answer the following questions.

If you circled NO, please move onto the next section, question 4.

- How many times have you lost consciousness?_____
- What is the duration of loss of consciousness?_____

4. Do you have any disabilities?

YES	NO

If you circled YES above (question 4), please answer the following question.

If you circled NO, please move onto the next section, SECTION A.

- Please indicate your disability:_____

The next few sections consist of a series of self-report scales. Please read the instructions for each scale carefully & answer each statement/question truthfully. There are no right or wrong answers for these. As previously stated, all information

Section 1: *(Resilience)**

Scale 1A *(Scale of Protective Factors)**

The following sentences describe how you feel about yourself. Read each statement carefully. Please circle a number next to each statement that most reflects your life. There are no right or wrong answers.

Key to indicate how you feel:

Disagree completely 1	Disagree moderately 2	Disagree somewhat 3	Neither disagree or agree 4	Agree somewhat 5	Agree moderately 6	Agree completely 7

1	I am good at starting new conversations	1	2	3	4	5	6	7
2	My friends and/or family, keep me up to speed on important events	1	2	3	4	5	6	7
3	I am good at making new friendships	1	2	3	4	5	6	7
4	My friends and/or family, are supportive of one another	1	2	3	4	5	6	7
5	When working on something, I make a list of things to do in order of importance	1	2	3	4	5	6	7
6	I am confident in my ability to solve problems	1	2	3	4	5	6	7
7	My friends and/or family, spend free time together	1	2	3	4	5	6	7
8	When working on something, I set priorities before I start	1	2	3	4	5	6	7
9	I am confident in my ability to succeed	1	2	3	4	5	6	7
10	I am confident in my ability to think out and plan	1	2	3	4	5	6	7
11	I am confident in my ability to think on my feet	1	2	3	4	5	6	7
12	I am good at working with others as part of a team	1	2	3	4	5	6	7
13	I am good at socializing with new people	1	2	3	4	5	6	7
14	I am confident in my ability to achieve goals	1	2	3	4	5	6	7
15	When working on something, I organize my time well	1	2	3	4	5	6	7
16	I am good at interacting with others	1	2	3	4	5	6	7
17	I am good at being with other people	1	2	3	4	5	6	7
18	When working on something, I plan things out	1	2	3	4	5	6	7
19	I am confident in my ability to make good decisions/choices	1	2	3	4	5	6	7

20	My friends and/or family see things the same way as I do	1	2	3	4	5	6	7
21	My friends and/or family are seen as united	1	2	3	4	5	6	7
22	When working on something, I do better if I set a goal	1	2	3	4	5	6	7
23	My friends and/or family are optimistic	1	2	3	4	5	6	7
24	When working on something, I can see the order in which to do things	1	2	3	4	5	6	7

Scale 1B *(Brief Resilience Scale)**

Please respond to each statement below by ticking ONE box ONLY. There are no right or wrong answers.

Key to indicate how you feel:

Not at all 1	A little 2	Somewhat 3	Quite a bit 4	Alot 5

1	I tend to bounce back quickly after hard times	1	2	3	4	5
2	I have a hard time making it through stressful events	5	4	3	2	1
3	It does not take me long to recover from a stressful event	1	2	3	4	5
4	It is hard for me to snap back when something bad happens	5	4	3	2	1
5	I usually come through difficult times with little trouble	1	2	3	4	5
6	I tend to take a long time to get over set-backs in life	5	4	3	2	1

Scale 1C *(Adult Resilience Measure)**

Please read the following carefully. To what extent do the sentences below describe you? Tick one answer for each statement.

Not at all 1	A little 2	Somewhat 3	Quite a bit 4	Alot 5

1	I have people I respect in my life	1	2	3	4	5
2	I cooperate with people around me	1	2	3	4	5
3	Getting and improving qualifications or skills is important to me	1	2	3	4	5
4	I know how to behave in different social situations	1	2	3	4	5
5	My family have usually supported me through life	1	2	3	4	5
6	My family know a lot about me	1	2	3	4	5
7	If I am hungry, I can get food and eat	1	2	3	4	5
8	I try to finish what I start	1	2	3	4	5
9	Spiritual beliefs are a source of strength for me	1	2	3	4	5
10	I am proud of my ethnic background	1	2	3	4	5
11	People think that I am fun to be with	1	2	3	4	5
12	I talk to my family/partner about how I feel	1	2	3	4	5

13	I can solve problems without harming myself or others (e.g without using drugs or being violent)	1	2	3	4	5
14	I feel supported by my friends	1	2	3	4	5
15	I know where to get help in my community	1	2	3	4	5
16	I feel I belong in my community	1	2	3	4	5
17	My family stands by me during difficult times	1	2	3	4	5
18	My friends stand by me during difficult times	1	2	3	4	5
19	I am treated fairly in my community	1	2	3	4	5
20	I have opportunities to show others that I can act responsibly	1	2	3	4	5
21	I am aware of my own strengths	1	2	3	4	5
22	I participate in organized religious activities	1	2	3	4	5
23	I think it is important to support my community	1	2	3	4	5
24	I feel secure when I am with my family	1	2	3	4	5
25	I have opportunities to apply my abilities in life (like skills, a job, caring for others)	1	2	3	4	5
26	I enjoy my family's/partner's culture and family traditions	1	2	3	4	5
27	I enjoy my community's culture and traditions	1	2	3	4	5
28	I am proud to be a citizen of South Africa	1	2	3	4	5

Section 2: *(Anxiety)**

Scale 2A *(Social Anxiety Questionnaire)**

Below are a series of social situations that may or may not cause you UNEASE, STRESS or NERVOUSNESS. Please place an "**X**" on the number next to each social situation that best reflects your reaction, where "1" represents no unease, stress or nervousness and "5" represents very high or extreme unease stress, or nervousness. If you have never experienced the situation described, please **imagine** what your level of UNEASE, STRESS, or NERVOUSNESS might be if you were in that situation, and rate how you imagine you would feel by placing an "**X**" on the corresponding number.

Key to indicate how you feel:

Not at all or very slight 1	Slight 2	Moderate 3	High 4	Very high or extremely high 5

Please rate all the items and do so **honestly**; do not worry about your answer because there are no right or wrong ones.

Greeting someone and being ignored	1	2	3	4	5
Having to ask a neighbour to stop making noise	1	2	3	4	5
Speaking in public	1	2	3	4	5
Asking someone attractive of the opposite sex for a date	1	2	3	4	5
Complaining to the waiter about my food	1	2	3	4	5
Feeling watched by people of the opposite sex	1	2	3	4	5
Participating in a meeting with people in authority	1	2	3	4	5
Talking to someone that isn't paying attention to what I am saying	1	2	3	4	5
Refusing when asked to something I don't like doing	1	2	3	4	5
Making new friends	1	2	3	4	5

Telling someone they have hurt my feelings	1	2	3	4	5
Having to speak in a class, at work, or in a meeting	1	2	3	4	5
Maintaining a conversation with someone I've just met	1	2	3	4	5
Expressing my annoyance at someone that is picking on me	1	2	3	4	5
Greeting each person at a social meeting when I don't know most of them	1	2	3	4	5
Being teased in public	1	2	3	4	5
Talking to people I don't know at a party or meeting	1	2	3	4	5
Being asked a question in class by a teacher or by a superior in a meeting	1	2	3	4	5
Looking into the eyes of someone I have just met while we are talking	1	2	3	4	5
Being asked out by a person I am attracted to	1	2	3	4	5
Making a mistake infront of other people	1	2	3	4	5
Attending a social event where I know only one person	1	2	3	4	5
Starting a conversation with someone of the opposite sex that I like	1	2	3	4	5
Being reprimanded about something I have done wrong	1	2	3	4	5
While having dinner with colleagues, classmates or workmates, being asked to speak on behalf of the entire group	1	2	3	4	5
Telling someone that their behaviour bothers me and asking them to stop	1	2	3	4	5
Asking someone I find attractive to dance	1	2	3	4	5
Being criticized	1	2	3	4	5
Talking to a superior or a person in authority	1	2	3	4	5
Telling someone I am attracted to that I would like to get to know them better	1	2	3	4	5

Scale 2B *(Spielberger State Trait Inventory)**

Part 1

A number of statements which people have used to describe themselves are given below. Read each statement then tick the appropriate number to the right statement to indicate how you feel *right now*, that is, *at this moment*. There are no right or wrong answers. Do not spend too much time on any one statement, but give the answer which describes your feelings at best.

Key to indicate how your feel:

Not at all 1	Somewhat 2	Moderately so 3	Very much so 4

1	I feel calm	1	2	3	4
2	I feel secure	1	2	3	4
3	I feel tense	1	2	3	4
4	I feel strained	1	2	3	4
5	I feel at ease	1	2	3	4
6	I feel upset	1	2	3	4
7	I am presently worrying over possible misfortunes	1	2	3	4
8	I feel satisfied	1	2	3	4
9	I feel frightened	1	2	3	4
10	I feel comfortable	1	2	3	4
11	I feel self-confident	1	2	3	4
12	I feel nervous	1	2	3	4
13	I feel jittery	1	2	3	4
14	I feel indecisive	1	2	3	4
15	I feel relaxed	1	2	3	4
16	I feel content	1	2	3	4
17	I am worried	1	2	3	4
18	I feel confused	1	2	3	4

19	I feel steady	1	2	3	4
20	I feel pleasant	1	2	3	4

Part 2

A number of statements which people have used to describe themselves are given below. Read each statement then tick the appropriate number to the right statement to indicate how you *generally feel*. There are no right or wrong answers. Do not spend too much time on any one statement, but give the answer which describes your feelings at best.

Key to indicate how your feel:

Not at all 1	Somewhat 2	Moderately so 3	Very much so 4

21	I feel pleasant	1	2	3	4
22	I feel nervous and restless	1	2	3	4
23	I feel satisfied with myself	1	2	3	4
24	I wish I could be as happy as others seem to be	1	2	3	4
25	I feel like a failure	1	2	3	4
26	I feel rested	1	2	3	4
27	I am "calm, cool and collected"	1	2	3	4
28	I feel that difficulties are piling up and I cannot overcome them	1	2	3	4
29	I worry too much over something that really doesn't matter	1	2	3	4
30	I am happy	1	2	3	4
31	I have disturbing thoughts	1	2	3	4
32	I lack self confidence	1	2	3	4
33	I feel secure	1	2	3	4
34	I make decisions easily	1	2	3	4
35	I feel inadequate	1	2	3	4
36	I am content	1	2	3	4
37	Some unimportant thought runs through my mind and bothers me	1	2	3	4
38	I take disappointments so keenly that I can't put them out of my mind	1	2	3	4
39	I am a steady person	1	2	3	4
40	I get in a state of tension or turmoil as I think over my recent concerns and interests	1	2	3	4

Scale 2C *(Beck Anxiety Inventory)**

Please carefully read each item in the list. Indicate how much you have been bothered by that symptom during the past month, including today, by ticking the number in the corresponding space in the column next to each symptom.

Key to indicate how you feel:

Not at all 1	Mildly, but it didn't bother me much 2	Moderately, it wasn't pleasant at times 3	Severely, it bothered me alot 4

1	Numbness or tingling	1	2	3	4
2	Feeling hot	1	2	3	4
3	Wobbliness in legs	1	2	3	4
4	Unable to relax	1	2	3	4
5	Fear of the worst happening	1	2	3	4
6	Dizzy or lightheaded	1	2	3	4

7	Heart pounding/racing	1	2	3	4
8	Unsteady	1	2	3	4
9	Terrified or afraid	1	2	3	4
10	Nervous	1	2	3	4
11	Feeling of choking	1	2	3	4
12	Hands trembling	1	2	3	4
13	Shaky/ unsteady	1	2	3	4
14	Fear of losing control	1	2	3	4
15	Difficulty in breathing	1	2	3	4
16	Fear of dying	1	2	3	4
17	Scared	1	2	3	4
18	Indigestion	1	2	3	4
19	Faint/lightheaded	1	2	3	4
20	Face flushed	1	2	3	4
21	Hot/cold sweats	1	2	3	4

Section 3: *(Childhood trauma)**

Scale 3A *(Adverse Childhood Experiences)**

Please tick the box of the appropriate number for each respective statement.
During your childhood (up until you matriculated from school) did you feel any of the following and to what extent?

Key to indicate how your feel:

Never true	Rarely true	Sometimes true	Often true	Very often true
1	2	3	4	5

I didn't have enough to eat.	1	2	3	4	5
I knew there was someone to take care of me and protect me.	1	2	3	4	5
People in my family called me things like "stupid", "lazy", or "ugly".	1	2	3	4	5
My parents were too drunk or high to take care of me.	1	2	3	4	5
There was someone in my family who helped me feel important or special.	1	2	3	4	5
I had to wear dirty clothes.	1	2	3	4	5
I felt loved.	1	2	3	4	5
I thought that my parents wished I had never been born.	1	2	3	4	5
I got hit so hard by someone in my family that I had to see a doctor or go to hospital.	1	2	3	4	5
There was nothing I wanted to change about my family.	1	2	3	4	5
People in my family hit me so hard that it left bruises or marks.	1	2	3	4	5
I was punished with a belt, a board, a cord, or some hard object.	1	2	3	4	5
People in my family looked out for each other.	1	2	3	4	5
People in my family said hurtful or insulting things to me.	1	2	3	4	5
I believe that I was physically abused.	1	2	3	4	5
I had the perfect childhood.	1	2	3	4	5
I go hit or beaten so badly that it was noticed by someone like a teacher, neighbor, or doctor.	1	2	3	4	5
I felt that someone in my family hated me.	1	2	3	4	5
People in my family felt close to each other.	1	2	3	4	5
Someone tried to touch me in a sexual way, or tried to make me touch them.	1	2	3	4	5
Someone threatened to hurt me or tell lies about me unless I did something sexual with them.	1	2	3	4	5
I had the best family in the world.	1	2	3	4	5
Someone tried to make me do sexual things or make me watch sexual things.	1	2	3	4	5
Someone molested me.	1	2	3	4	5
I believe that I was emotionally abused.	1	2	3	4	5
There was someone to take me to the doctor if I needed it.	1	2	3	4	5

I believe that I was sexually abused	1	2	3	4	5
My family was a source of strength and support.	1	2	3	4	5

Scale 3B *(Bernstein Childhood Trauma Questionnaire)**

Please tick 'Yes" OR 'No" for the corresponding statement. There are no right or wrong answers.
While you were growing up, during your first 18 years of life:

		Yes	No
1	Did your parent or any other adult in the household **often**:		
	Swear at you, insult you or put you down? **Or**		
	Humiliate you? **Or**		
	Act in a way that made you afraid that you might be physically hurt?		
2	Did a parent or other adult in the household **often**:		
	Push, grab, slap or throw something at you? **Or**		
	Ever hit you so hard that you had marks or were injured?		
3	Did an adult or person at least 5 years older than you **ever**:		
	Touch or fondle you or have you touch their body in a sexual way? **Or**		
	Try to or actually have oral, anal, or vaginal sex with you?		
4	Did you **often** feel that:		
	No one in your family loved you or thought you were important or special? **Or**		
	Your family didn't look out for each other, feel close to each other or support each other?		
5	Did you **often** feel that:		
	You didn't have enough to eat, had to wear dirty clothes and had no one to protect you?		
	Your parents were too drunk or high to take care of you or to take you to the doctor if you needed it?		
6	Were your parents ever separated or divorced?		
7	Was your mother or stepmother often pushed, grabbed, slapped or had something thrown at her? or Sometimes or often kicked, bitten, hit with a fist or hit with something hard? Or Ever repeatedly hit over at least a few minutes or threatened with a gun or knife?		
8	Did you live with anyone who was a problem drinker or alcoholic or who used street drugs?		
9	Was a household member depressed or mentally ill or did a household member attempt suicide?		
10	Did a household member go to prison?		

Thank you for participating in this research study.

Should you have any questions, please feel free to contact either of the contact persons provided on the cover page.

Appendix V

Table 2.1 Raw data of finalized scoring across scales for full sample group (*N*=54)

Sample ID	Sex	Age	SPF SUB 1	SPF SUB 2	SPF SUB 3	SPF SUB 4	TOT. SPF	TOT. BRS	ARM SUB 1	ARM SUB 2	ARM SUB 3	TOT. ARM	SAQ SUB 1	SAQ SUB 2	SAQ SUB 3	SAQ SUB 4	SAQ SUB 5	TOT. SAQ	TOT. ST	TOT. TR	TOT. BAI	CTQ SUB 1	CTQ SUB 2	CTQ SUB 3	CTQ SUB 4	CTQ SUB 5	CTQ TOT.	CTQ DEN.	TOT. ACE
239	F	19	4.17	6.83	5.5	5.67	22.17	2.83	4.27	4.86	4.3	13.43	22	29	18	18	22	109	22	22	42	7	6	5	5	5	28	27	0
156	F	23	2.17	4.67	5.83	4.33	17	2.17	4	4.29	3.9	12.19	24	27	23	23	27	124	19	18	39	11	6	8	9	6	40	38	-
175	F	21	3.33	4.5	5.5	3.33	16.67	3.33	3.09	3.71	3.4	10.2	18	21	16	16	19	90	20	21	50	11	7	7	11	8	44	43	4
188	F	22	4.5	5.33	6.33	5.67	21.83	3.33	4.36	4.29	4.3	12.95	11	10	7	7	15	50	32	24	78	23	5	17	11	9	65	65	7
149	F	21	3.5	4.5	6.33	6.17	20.5	3	3.64	3.57	3.7	10.91	22	23	13	13	18	89	21	21	29	8	8	5	13	13	47	47	2
86	F	20	5.67	5.33	6.83	7	24.83	2.5	4.45	4	5	13.45	22	24	17	17	17	97	25	16	38	11	5	5	11	5	37	37	1
66	F	22	4.67	4.67	4.5	3.83	17.67	2.5	4.09	3.86	3.8	11.75	18	29	16	16	24	103	38	33	60	7	5	5	15	13	45	45	7
159	M	22	3.67	2.67	4.5	3.83	14.67	3.33	3.09	3.14	1.8	8.03	20	22	20	20	15	97	19	15	29	9	6	5	11	8	39	39	2
15	F	20	4.17	3.83	5.17	5.33	18.5	3.33	4.55	4.71	4.1	13.36	28	27	23	23	23	124	26	26	46	5	6	5	6	5	27	27	1
65	M	21	4	5.67	5	4.5	19.17	3.5	3.82	3.86	2.9	10.58	18	18	22	22	22	102	21	17	63	16	14	15	16	13	74	74	0
238	F	20	3	2.67	3.83	3.33	12.83	2.83	4.09	4.14	3.6	11.83	13	20	15	15	15	78	22	18	43	8	5	5	10	6	34	33	-
194	M	22	4.33	6.17	6.33	3.5	20.33	3.17	4.45	5	4.8	14.25	14	22	18	18	23	95	17	13	37	5	7	5	6	5	28	28	0
83	M	21	3.33	5.33	4.5	3.83	17	2.83	3.91	3.57	3.5	10.98	22	19	18	18	21	98	19	26	35	7	7	5	12	8	39	39	0
90	F	21	3.83	4.33	6.67	7	21.83	3.33	5	4.86	5	14.86	20	22	28	28	23	121	26	25	39	7	7	5	5	9	33	32	1
103	F	22	5.17	3.83	6	6	21	1.67	3.91	4.86	4.4	13.17	24	26	20	20	28	118	18	17	27	5	5	5	5	5	25	23	1
169	F	22	6.5	5.67	5	5.17	22.33	3.83	4.55	4.29	4.5	13.34	24	25	21	21	22	113	34	30	56	8	8	5	6	9	36	36	2
74	F	22	4	4	5	3.17	16.17	3	3.45	2.71	2.4	8.56	19	18	20	20	21	98	19	21	36	6	5	5	15	6	37	37	0
172	F	22	4.33	5	6	5.17	20.5	2.83	4.27	4.57	3.1	11.94	30	30	29	29	28	146	35	31	62	6	6	5	9	9	35	35	1
85	M	20	4.5	3.5	5.5	2.33	15.83	3.83	4.27	2.71	3.2	10.18	20	24	28	28	26	126	21	25	54	16	9	19	11	9	64	64	7
94	M	21	5.67	5.83	5.5	4.5	21.5	2.83	4.27	4.43	4.7	13.4	18	20	21	21	19	99	15	18	34	5	5	5	7	7	29	29	1
165	F	23	2.5	5.33	5.5	5.33	18.67	3.33	4.36	4.86	4.1	13.32	25	16	28	28	25	122	30	26	53	5	5	5	6	10	31	29	0
125	F	23	5.5	4.67	5.83	6	22	3	4.36	4.57	4.2	13.13	22	18	23	23	18	104	26	22	49	16	5	16	10	6	53	53	7
53	F	21	4.83	4.5	6.33	6.5	22.17	3.67	4.73	5	4.5	14.23	29	26	29	29	23	136	26	20	42	5	6	5	5	5	26	25	1
151	F	22	4	5.17	6.83	6	22	3	4.64	4.29	2.9	11.83	27	22	11	11	18	89	15	21	74	13	6	8	5	5	37	37	2
95	F	22	6.33	5.33	6.67	6.83	25.17	2.33	4.91	5	4.5	14.41	9	20	10	10	6	55	11	11	37	9	7	5	8	5	34	34	4
193	F	20	5	5.67	6	6.5	23.17	2.5	4.18	3.71	4.1	11.99	23	29	26	26	18	122	30	26	61	16	7	10	9	7	49	47	7
71	M	19	4.83	5.33	5.83	5.17	21.17	2.83	4.18	4.57	3.3	12.05	18	18	18	18	18	90	26	19	42	5	5	5	7	6	28	28	0

031 OS	M	24	4.83	5.17	6.33	3.5	19.83	1.5	4	4.86	4	12.86	19	18	15	15	18	85	12	11	30	7	7	5	7	5	31	30	1
184	F	20	6	5.5	5.33	4.67	21.5	2	4.45	4.71	3.9	13.06	11	20	23	23	19	96	17	20	38	6	8	5	9	6	34	34	2
131	F	22	4.5	5.33	4.5	5.33	19.67	3	3.45	3.86	4	11.31	29	23	24	24	29	129	29	24	44	9	5	5	5	5	29	28	0
57	F	19	4.33	1.5	6	3.17	15	4.17	3.09	2.29	4.1	9.48	23	20	18	18	9	88	25	20	23	9	9	5	16	7	46	46	0
128	F	23	5.5	5.5	6	4.17	21.17	2.83	3.36	4	3.1	10.46	14	19	16	16	16	81	21	22	30	6	6	5	13	8	38	38	4
96	F	21	6.33	5.17	5.83	6.33	23.67	2.83	4	4.29	3.3	11.59	25	30	26	26	24	131	16	14	34	7	6	9	6	5	33	33	2
134	F	22	3.17	5	4.33	3.83	16.33	2.67	3.73	3.29	2.9	9.92	29	29	24	24	25	131	33	30	51	14	7	6	12	7	46	46	6
013 OS	F	24	3.67	4.83	4	5.33	17.83	3.17	4	4.86	4.1	12.96	26	27	19	19	24	115	14	25	40	13	5	5	13	7	43	43	5
111	M	21	2.17	4	6	3.67	15.83	2.67	4.18	4	3.6	11.78	16	24	17	17	23	97	21	19	29	8	8	5	12	6	39	39	5
82	F	21	4	4.83	5	4.17	18	2.67	4.27	4.71	3.6	12.58	19	21	20	20	19	99	17	20	37	10	7	5	6	9	37	37	0
5	F	21	4.33	5.83	6.33	5.5	22	3	4.27	5	4.7	13.97	21	21	20	20	18	100	11	14	29	5	6	5	5	5	26	26	5
25	F	26	2	3.5	5.67	6.33	17.5	3.17	3.73	3.29	2.9	9.92	30	30	22	22	30	134	24	27	26	23	5	5	15	7	55	55	4
109	M	19	5.83	5.17	6.17	5.33	22.5	3	4.27	4.43	4.6	13.3	9	10	10	10	9	48	11	14	21	6	5	5	5	9	30	28	0
105	F	23	2	5.17	5	2.83	15	3.33	3.73	3.86	4.1	11.69	24	26	8	8	27	93	29	23	27	8	5	5	10	7	35	35	4
91	M	22	3.5	4.17	5.5	5.17	18.33	3.33	3.45	3.29	3.5	10.24	20	18	17	17	17	89	26	24	51	6	9	6	10	6	37	37	1
185	F	20	5.83	6.33	5.67	5	22.83	3	4.64	4.86	4.6	14.1	23	28	12	12	10	85	13	15	50	5	5	5	6	5	26	24	0
35	F	19	3.83	5.83	5.83	5.33	20.83	3.17	4.27	4.43	4.2	12.9	20	22	23	23	13	101	21	20	33	5	5	5	5	5	25	24	0
73	F	20	4.33	5.83	4.33	5	19.5	2.33	3.73	4.86	4.2	12.79	26	25	16	16	22	105	15	11	28	5	6	5	5	5	26	23	0
24	M	20	6.17	5.5	7	5.17	23.83	3.67	5	5	4.4	14.4	26	16	30	30	14	116	12	13	21	9	11	5	7	9	41	41	7
4	F	21	3.83	5.17	3.33	3.17	15.5	3	3.18	3	3.8	9.98	28	26	23	23	24	124	32	28	38	9	5	5	7	11	37	35	0
44	F	20	5.5	4.67	5.33	6	21.5	3.17	4.45	3.86	4.3	12.61	25	30	27	27	15	124	30	24	31	10	5	5	16	8	44	44	8
92	M	21	5	5.5	6.83	6.17	23.5	2.5	4.27	4.43	4.4	13.1	20	20	19	19	19	97	20	15	35	7	5	5	6	6	29	29	2
139	M	22	6.83	6.5	6.83	6.67	26.83	3.17	4.91	5	5	14.91	21	22	10	10	20	83	18	19	51	11	8	5	8	8	40	40	2
49	M	21	5.67	5.17	5	5.33	21.17	2.83	4.91	4.86	4.2	13.97	18	22	18	18	24	100	12	12	23	5	9	5	9	9	37	37	0
70	M	21	7	6.67	6.83	6.67	27.17	2.83	4.82	4.29	4.9	14.01	17	15	11	11	7	61	23	16	50	9	7	5	10	5	36	36	3
84	M	27	3.33	3	6.17	4.67	17.17	2.33	4.18	3.71	4.4	12.29	24	9	12	12	16	73	14	13	28	6	5	7	18	12	48	48	-
235	F	19	1.83	5.33	3.5	4	14.67	3.33	3.55	4.29	3.8	11.64	30	30	18	18	30	126	37	36	78	13	13	9	8	5	48	47	-

Table 2.2 Data distribution testing with Shapiro-Wilks test (SW) with p-value (P)) for all scales and sub dimensions for total sample population

Scale	*N*	SW	*P*
SPF sub 1	54	0.98	0.68
SPF sub 2	54	0.94	0.13
SPF sub 3	54	0.96	0.46
SPF sub 4	54	0.96	0.11
Total SPF	54	0.97	0.26
Total BRS	54	0.97	0.13
ARM sub 1	54	0.96	0.11
ARM sub 2	54	0.92	0.00
ARM sub 3	54	0.96	0.04
Total ARM	54	0.97	0.01
SAQ sub 1	54	0.97	0.12
SAQ sub 2	54	0.95	0.03
SAQ sub 3	54	0.98	0.35
SAQ sub 4	54	0.98	0.35
SAQ sub 5	54	0.97	0.21
Total SAQ	54	0.96	0.11
Total State	54	0.96	0.10
Total Trait	54	0.98	0.32
Total BAI	54	0.93	0.01
CTQ sub 1	54	0.82	0.00
CTQ sub 2	54	0.77	0.00
CTQ sub 3	54	0.48	0.00
CTQ sub 4	54	0.91	0.00
CTQ sub 5	54	0.85	0.00
Total CTQ	54	0.91	0.00
Total ACE[a]	50	0.83	0.00

For ACE n=50 due to the removal of incomplete responses

Table 2.3 Subjective questionnaire and scale scoring for all scales and sub-scales for the total samples and by sex.

Scale	*Total sample group (N=54)*					*Males (N=17)* [a]					*Females (N=34)* [b]						
	Mean	**Std.Dev.**	**Median**	**Min**	**Max**	**Mean**	**Std.Dev.**	**Median**	**Min**	**Max**	**Mean**	**Std.Dev.**	**Median**	**Min**	**Max**	**t**	**z**
SPF sub 1	4.52	1.2	4.415	2	7	4.84	1.29	4.83	2.17	7	4.37	1.15	4.33	2	6.5	-1.36	-
SPF sub 2	4.99	1.01	5.17	1.5	6.83	5.06	1.11	5.33	2.67	6.67	4.96	0.98	5.17	1.5	6.83	-0.31	-
SPF sub 3	5.58	0.9	5.75	3.33	7	5.87	0.8	6	4.5	7	5.45	0.92	5.67	3.33	6.83	-	-1.53
SPF sub 4	5.03	1.18	5.185	2.33	7	4.76	1.21	5.17	2.33	6.67	5.16	1.16	5.33	2.83	7	1.15	-
Total SPF	20.13	3.06	20.5	14.67	27.17	20.53	3.6 *	20.37	14.67	27.17	19.94	2.81	20.5	15	25.17	-0.65	-
Total BRS	2.95	0.51	3	1.5	4.17	2.95	0.55	2.83	1.5	3.83	2.95	0.49	3	1.67	4.17	0.00	
ARM sub 1	4.13	0.5	4.225	3.09	5	4.23	0.51	4.27	3.09	5	4.08	0.5	4.18	3.09	5	-1.08	-
ARM sub 2	4.2	0.68	4.29	2.29	5	4.19	0.71	4.43	2.71	5	4.21	0.68	4.29	2.29	5	-	-0.04
ARM sub 3	3.94	0.68	4.1	1.8	5	3.95	0.85	4.2	1.8	5	3.93	0.61	4.1	2.4	5	-	-0.53
Total ARM	114.15	14.79	116.5	74	139	115.41	17.29 *	118	74	139	113.57	13.72	116	81	139	-0.42	-
SAQ sub 1	21.35	5.34	22	9	30	18.82	3.81 #	19	9	26	22.51	5.58	23	9	30	2.47	-
SAQ sub 2	22.33	5.21	22	9	30	18.65	4.3	19	9	24	24.03	4.72	25	10	30	-	3.60
SAQ sub 3	19.19	5.76	19	7	30	17.88	5.58	18	10	30	19.78	5.82	20	7	29	1.13	-
SAQ sub 4	19.19	5.76	19	7	30	17.88	5.58	18	10	30	19.78	5.82	20	7	29	1.13	-
SAQ sub 5	19.91	5.68	19	6	30	18.29	5.07	19	7	26	20.65	5.86	22	6	30	1.43	-
Total SAQ	101.96	21.81	99.5	48	146	91.53	18.48 #	97	48	126	106.76	21.76	105	50	146	2.50	-
Total State	21.96	7.18	21	11	38	18.06	4.8 #	19	11	26	23.76	7.43	24	11	38	2.89	-
Total Trait	20.57	5.91	20	11	36	17	4.57 #	16	11	26	22.22	5.77	22	11	36	3.28	-
Total BAI	41.31	13.98	38	21	78	37.24	12.58 #	35	21	63	43.19	14.35	39	23	78	-	1.4
CTQ sub 1	8.91	4.27	8	5	23	8.06	3.45 #	7	5	16	9.3	4.59	8	5	23	0.87	-
CTQ sub 2	6.57	1.98	6	5	14	7.47	2.45 *	7	5	14	6.16	1.59	6	5	13	-2.1	-
CTQ sub 3	6.33	3.22	5	5	19	6.59	4.02 *	5	5	19	6.22	2.84	5	5	17	0.05	-

CTQ sub 4	9.13	3.61	9	5	18	9.53	3.57	9	5	18	8.95	3.66	9	5	16	-0.8	-
CTQ sub 5	7.2	2.28	7	5	13	7.71	2.34	8	5	13	6.97	2.24	6	5	13	-1.27	-
Total CTQ	37.67	10.72	37	23	74	39.18	12.74 *	37	28	74	36.97	9.78	36	23	65	-0.6	-
Total ACE	2.38	2.54	1.5	0	8	1.94	2.41	1	0	7	2.59	2.61	2	0	8	0.79	-

[a] For ACE, males (n=16); females (n=31)

Table 2.4 Parametric scale data correlates for population and by sex, significant correlations are highlighted in read where p-value < 0.05.

		Mean			Std Dev			r^2			t			p		
		All	*Male*	*Fem*	*All*	*Male*	*Fem*	*All*	*Male*	*Fem*	*All*	*Male*	*Fem*	*All*	*Male*	*Fem*
SPF	*BRS*	2.95	2.95	2.95	0.51	0.55	0.49	0.04	0.01	0.07	-1.56	-0.45	-1.68	0.13	0.66	0.10
	ARM	114.15	115.41	113.57	14.79	17.29	13.72	0.54	0.69	0.45	7.86	5.78	5.31	0.00	0.00	0.00
	SAQ	101.96	91.53	106.46	21.81	18.48	21.76	0.05	0.20	0.01	-1.73	-1.95	-0.66	0.09	0.07	0.51
	STATE	21.96	18.16	23.76	7.18	4.80	7.43	0.07	0.03	0.08	-1.90	-0.72	-1.70	0.06	0.48	0.10
	TRAIT	20.57	17.00	22.22	5.91	4.57	5.77	0.14	0.14	0.15	-2.93	-1.53	-2.51	0.01	0.15	0.02
BRS	*ARM*	114.15	115.41	113.57	14.79	17.29	13.72	0.02	0.15	0.0.1	-1.10	-0.88	-0.63	0.28	0.39	0.53
	SAQ	101.96	91.53	106.76	21.81	18.48	21.76	0.02	0.18	0.00	1.03	1.84	0.20	0.31	0.09	0.84
	STATE	21.96	18.06	23.76	7.18	4.80	7.43	0.08	0.08	0.12	2.14	1.11	2.04	0.04	0.28	0.05
	TRAIT	20.57	17.00	22.22	5.91	4.57	5.77	0.10	0.16	0.11	2.43	1.72	2.10	0.02	0.11	0.04
ARM	*SAQ*	101.96	91.53	106.76	21.81	18.48	21.76	0.02	0.18	0.01	-1.10	-1.13	-0.44	0.28	0.28	0.66
	STATE	21.96	18.06	23.76	7.18	4.80	7.43	0.06	0.20	0.03	-1.84	-1.92	-1.12	0.07	0.07	0.27
	TRAIT	20.57	17.00	22.22	5.91	4.57	5.78	0.10	0.19	0.08	-2.40	-1.89	-1.72	0.02	0.08	0.09
SAQ	*STATE*	21.96	18.06	23.76	7.18	4.80	7.43	0.17	0.02	0.14	3.23	0.50	2.41	0.00	0.62	0.02
	TRAIT	20.57	17.00	22.22	5.91	4.57	5.77	0.24	0.09	0.20	4.06	1.24	2.95	0.00	0.23	0.01
STATE	*TRAIT*	20.57	17.00	22.22	5.91	4.57	5.77	0.69	0.42	0.71	10.85	3.31	9.21	0.00	0.00	0.00

Table 2.5 Non-parametric scale data correlates for population and by sex (***N***=54; excluding ACE, where ***N***=50), significant correlations are highlighted in read where p-value < 0.05.

		Spearman			t(N-2)			p-value		
		All	*Male*	*Fem*	*All*	*Male*	*Female*	*All*	*Male*	*Fem*
SPF	*ACE*[a]	0.12	0.05	0.15	0.85	-1.51	0.85	0.40	0.15	0.40
	CTQ	-0.26	-0.31	-0.23	-1.97	-1.28	-1.37	0.05	0.22	0.18
	BAI	0.06	-0.36	0.25	0.46	0.20	1.52	0.65	0.85	0.14
BRS	*ACE*[a]	0.03	0.17	-0.04	0.21	0.65	-0.24	0.84	0.52	0.81
	CTQ	0.21	0.39	0.12	1.52	1.62	0.72	0.13	0.13	0.48
	BAI	0.19	0.02	0.22	1.42	0.06	1.33	0.16	0.95	0.19
ARM	*ACE*[a]	-0.02	0.07	-0.03	-0.15	0.26	-0.15	0.88	0.80	0.88
	CTQ	-0.45	-0.29	-0.49	-3.63	-1.17	-3.32	0.00	0.26	0.00
	BAI	-0.02	-0.22	0.26	-0.13	-0.89	1.60	0.90	0.39	0.12
STATE	*ACE*[a]	0.16	0.17	0.18	1.13	0.65	1.04	0.27	0.53	0.31
	CTQ	0.24	0.10	0.37	1.82	0.38	2.34	0.07	0.71	0.03
	BAI	0.53	0.12	0.21	4.46	0.48	1.24	0.00	0.64	0.22
TRAIT	*ACE*[a]	0.22	0.13	0.27	1.56	0.51	1.59	0.12	0.62	0.12
	CTQ	0.34	0.22	0.46	2.62	0.88	3.04	0.01	0.39	0.00
	BAI	0.53	0.14	0.22	4.52	0.53	1.31	0.00	0.60	0.20
BA	*CTQ*	0.20	0.35	0.02	1.46	1.46	0.12	0.15	0.16	0.91

	ACE[a]	0.13	-0.24	0.09	0.89	-0.92	0.49	0.38	0.37	0.63	
CTQ	*ACE*[a]	0.55	0.46	0.61	4.57	1.92	4.36	0.00	0.07	0.00	

[a] *For ACE (n=50)*

Table 2.6 Parametric correlation data of all sub dimensions within scales (***N***=54), significant correlations are highlighted in read where p-value < 0.05

		Mean			**Std. Dev**			**r^2**			**t**			**p**		
		All	*Male*	*Fem*	*All*	*Male*	*Fem*	*All*	*Male*	*Fem*	*All*	*Male*	*Fem*	*All*	*Male*	*Fem*
SPF 1	*SPF 2*	4.99	5.06	4.96	1.01	1.12	0.98	0.09	0.35	0.02	2.25	2.84	0.77	0.03	0.01	0.48
	SPF 4	5.03	4.76	5.16	1.18	1.21	1.16	0.16	0.43	0.12	3.09	3.34	2.03	0.00	0.00	0.05
	ARM 1	4.13	4.23	4.08	0.50	0.51	0.50	0.27	0.51	0.16	4.35	3.96	2.56	0.00	0.00	0.02
	SAQ 1	21.35	18.82	22.51	5.34	3.81	5.58	0.10	0.00	0.15	-2.38	0.12	-2.46	0.02	0.91	0.02
	SAQ 3	19.19	17.88	19.78	5.76	5.58	5.82	0.01	0.05	0.00	-0.62	-0.91	0.13	0.54	0.38	0.89
	SAQ 4	19.19	17.88	19.78	5.76	5.58	582.00	0.01	0.05	0.00	-0.62	-0.91	0.13	0.54	0.38	0.89
	SAQ 5	19.91	18.29	20.65	5.68	5.07	5.86	0.26	0.23	-3.37	-4.22	-2.12	-3.37	0.00	0.05	0.00
SPF 2	*SPF 4*	5.03	4.76	5.16	1.18	1.21	1.16	0.08	0.23	0.04	2.17	2.13	1.24	0.03	0.05	0.22
	ARM 1	4.13	4.23	4.08	0.50	0.51	0.50	0.15	0.40	0.26	3.04	3.19	1.59	0.00	0.00	0.12
	SAQ 1	21.35	18.82	22.51	5.34	3.81	5.58	0.00	0.04	0.00	-0.43	-0.78	0.01	0.67	0.45	0.99
	SAQ 3	19.19	17.88	19.78	5.76	5.58	5.82	0.02	0.06	-0.08	-0.96	-0.94	-0.45	0.34	0.36	0.66
	SAQ 4	19.19	17.88	19.78	5.76	5.58	5.82	0.02	0.06	-0.08	-0.96	-0.94	-0.45	0.34	0.36	0.66
	SAQ 5	19.91	18.29	20.65	5.68	5.07	5.86	0.00	0.02	0.09	0.04	-0.60	0.51	0.97	0.56	0.61
SP F 4	*ARM 1*	4.13	4.23	4.08	0.50	0.51	0.50	0.30	0.19	0.43	4.7	1.85	5.11	0.00	0.08	0.00

	SAQ 1	21.35	18.82	22.51	5.34	3.81	5.58	0.02	0.01	0.01	0.89	0.33	0.45	0.38	0.75	0.65
	SAQ 3	19.19	18.65	24.03	5.76	4.30	4.72	0.00	0.20	0.01	0.18	-1.95	0.62	0.86	0.07	0.53
	SAQ 4	19.19	17.88	19.78	5.76	5.58	5.52	0.00	0.27	0.05	0.18	-2.33	1.39	0.86	0.03	0.17
	SAQ 5	19.91	18.29	20.65	5.68	5.07	5.86	0.03	0.30	0.00	-1.16	-2.54	-0.33	0.25	0.22	0.75
ARM 1	*SAQ 1*	21.35	18.82	22.51	5.34	3.81	5.58	0.03	0.00	0.03	-1.21	0.11	-1.05	0.23	0.91	0.30
	SAQ 3	19.19	17.88	19.78	5.76	5.58	5.82	0.00	0.00	0.01	0.31	-0.19	0.70	0.76	0.85	0.50
	SAQ 4	19.19	17.88	19.78	5.76	5.58	5.82	0.00	0.00	0.01	0.31	-0.19	0.70	0.76	0.85	0.50
	SAQ 5	19.91	18.29	20.65	5.68	5.07	5.86	0.04	0.00	0.06	-1.54	-0.15	-1.50	0.13	0.89	0.14
SAQ 1	*SAQ 3*	19.19	17.88	19.78	5.76	5.58	5.82	0.22	0.17	0.21	3.79	1.74	3.06	0.00	0.10	0.0
	SAQ 4	19.19	17.88	19.78	5.76	5.58	5.85	0.22	0.17	0.21	3.79	1.74	3.06	0.00	0.10	0.0
	SAQ 5	19.91	18.29	20.65	5.68	5.07	5.86	0.31	0.02	0.40	4.79	0.53	4.87	0.00	0.60	0.0
SAQ 3	*SAQ 4*	19.19	17.88	19.78	5.76	5.58	5.82	1.00	1.00	1.00						
	SAQ 5	19.91	18.29	20.65	5.68	5.07	5.86	0.18	0.17	0.16	3.36	1.76	2.59	0.00	0.10	0.01
SAQ 4	SAQ 5	19.91	18.29	20.65	5.68	5.07	5.86	0.18	0.17	0.16	0.36	1.76	2.59	0	0.10	0.01

Table 2.7 Non-parametric data of all sub dimensions within scales (***N***=54), significant correlations where p-value < 0.05

		Spearman			t(N-2)			p-value		
		All	*Male*	*Fem*	*All*	*Male*	*Fem*	***All***	***Male***	***Fem***
SPF 1	*SPF 3*	0.39	0.62	0.27	3.08	3.07	1.64	0.00	0.01	0.11
	ARM 2	0.37	0.56	0.24	2.90	2.64	1.46	0.01	0.02	0.15
	ARM 3	0.53	0.72	0.40	4.50	4.07	2.55	0.00	0.00	0.02
	SAQ 2	-0.21	-0.38	-0.09	-1.54	-1.60	-0.56	0.13	0.13	0.58
	CTQ 1	-0.17	-0.02	-0.20	-1.23	-0.07	-1.18	0.22	0.95	0.24
	CTQ 2	-0.03	-0.16	0.00	-0.18	-0.64	0.02	0.86	0.53	0.99
	CTQ 3	-0.13	-0.28	-0.06	-0.91	-1.15	-0.33	0.36	0.27	0.74
	CTQ 4	-0.19	-0.46	-0.09	-1.39	-2.02	-0.53	0.17	0.06	0.60
	CTQ 5	-0.11	0.10	-0.25	-0.83	0.39	-1.52	0.41	0.70	0.14
SPF 2	*SPF 3*	0.15	0.47	-0.02	1.09	2.06	-0.10	0.28	0.06	0.92
	ARM 2	0.44	0.61	0.31	3.56	2.95	1.94	0.00	0.01	0.06
	ARM 3	0.42	0.65	0.29	3.30	3.30	1.76	0.00	0.00	0.09
	SAQ 2	-0.03	-0.15	0.09	-0.20	-0.59	0.53	0.84	0.57	0.60
	CTQ 1	-0.11	-0.02	-0.10	-0.81	-0.06	-0.58	0.42	0.95	0.56
	CTQ 2	0.10	0.02	0.12	0.73	0.07	0.72	0.47	0.94	0.47
	CTQ 3	-0.04	-0.36	0.16	-0.29	-1.51	0.98	0.78	0.15	0.33
	CTQ 4	-0.40	-0.39	-0.40	-3.12	-1.66	-2.62	0.00	0.12	0.01
	CTQ 5	-0.27	-0.29	-0.29	-2.00	-1.18	-1.80	0.05	0.25	0.08
SPF 3	*ARM 2*	0.36	0.62	0.24	2.75	3.10	1.44	0.01	0.01	0.16
	ARM 3	0.47	0.71	0.33	3.87	3.93	2.10	0.00	0.00	0.04
	SAQ 2	-0.31	-0.29	-0.24	-2.36	-1.19	-1.45	0.02	0.25	0.15
	CTQ 1	-0.04	0.09	-0.04	-0.32	0.36	-0.22	0.75	0.72	0.83

	CTQ 2	0.09	-0.10	0.15	0.64	-0.40	0.92	0.53	0.69	0.36
	CTQ 3	-0.02	-0.29	0.12	-0.11	-1.19	0.74	0.91	0.25	0.46
	CTQ 4	-0.19	-0.51	-0.13	-1.43	-2.32	-0.81	0.16	0.04	0.43
	CTQ 5	-0.16	-0.33	-0.14	-1.18	-1.37	-0.82	0.24	0.19	0.42
SPF 4	*SPF 3*	0.55	0.42	0.42	4.74	1.77	1.77	0.00	0.10	0.10
	ARM 2	0.37	0.24	0.24	2.88	0.97	0.97	0.01	0.35	0.35
	ARM 3	0.48	0.52	0.52	3.98	2.36	2.36	0.00	0.03	0.03
	SAQ 2	0.08	-0.46	-0.46	0.56	-2.03	-2.03	0.57	0.06	0.06
	CTQ 1	0.07	-0.06	-0.06	0.47	-0.21	-0.21	0.64	0.83	0.83
	CTQ 2	-0.16	-0.21	-0.21	-1.14	-0.85	-0.85	0.26	0.41	0.41
	CTQ 3	0.02	-0.15	-0.15	0.17	-0.60	-0.60	0.87	0.56	0.56
	CTQ 4	-0.28	-0.14	-0.14	-2.09	-0.54	-0.54	0.04	0.59	0.59
	CTQ 5	-0.21	0.13	0.13	-1.55	0.52	0.52	0.13	0.61	0.61
ARM 1	*ARM 2*	0.36	0.68	0.64	2.75	3.64	4.90	0.01	0.00	0.00
	ARM 3	0.47	0.74	0.57	3.87	4.21	4.12	0.00	0.00	0.00
	SPF 3	0.50	0.62	0.42	4.17	3.09	2.75	0.00	0.01	0.01
	SAQ 2	-0.31	0.06	-0.05	-2.36	0.24	-0.30	0.02	0.82	0.77
	CTQ 1	-0.04	-0.05	-0.17	-0.32	-0.21	-1.03	0.75	0.84	0.31
	CTQ 2	0.09	0.11	0.00	0.64	0.45	-0.02	0.53	0.66	0.99
	CTQ 3	-0.02	-0.35	-0.07	-0.11	-1.43	-0.40	0.91	0.17	0.69
	CTQ 4	-0.19	-0.47	-0.39	-1.43	-2.07	-2.47	0.16	0.06	0.02
	CTQ 5	-0.16	0.02	-0.19	-1.18	0.07	-1.12	0.24	0.95	0.27
ARM 2	*SPF 3*	0.55	0.62	0.24	4.74	3.10	1.44	0.00	0.01	0.16
	ARM 3	0.48	0.63	0.58	3.98	3.17	4.18	0.00	0.01	0.00
	SAQ 2	0.08	-0.05	-0.09	0.56	-0.20	-0.53	0.57	0.85	0.60
	CTQ 1	0.07	-0.31	-0.48	0.47	-1.24	-3.23	0.64	0.23	0.00
	CTQ 2	-0.16	-0.01	0.03	-1.14	-0.04	0.16	0.26	0.97	0.88
	CTQ 3	0.02	-0.60	-0.18	0.17	-2.90	-1.10	0.87	0.01	0.28

	CTQ 4	-0.28	-0.69	-0.66	-2.09	-3.65	-5.24	0.04	0.00	0.00
	CTQ 5	-0.21	-0.24	-0.40	-1.55	-0.97	-2.61	0.13	0.35	0.01
ARM 3	*SPF 3*	0.47	0.71	0.33	3.87	3.93	2.10	0.00	0.00	0.04
	ARM 2	0.60	0.63	0.58	5.38	3.17	4.18	0.00	0.01	0.00
	SAQ 2	-0.12	-0.19	-0.09	-0.85	-0.76	-0.51	0.40	0.46	0.61
	CTQ 1	-0.27	-0.24	-0.26	-2.01	-0.96	-1.57	0.05	0.35	0.13
	CTQ 2	-0.12	-0.26	-0.09	-0.89	-1.04	-0.53	0.38	0.32	0.60
	CTQ 3	-0.31	-0.43	-0.23	-2.33	-1.87	-1.37	0.02	0.08	0.18
	CTQ 4	-0.42	-0.50	-0.39	-3.32	-2.25	-2.53	0.00	0.04	0.02
	CTQ 5	-0.20	-0.26	-0.27	-1.49	-1.05	-1.67	0.14	0.31	0.10
SAQ 1	*SPF 3*	-0.19	0.03	-0.25	-1.43	0.11	-1.51	0.16	0.91	0.14
	ARM 2	-0.06	-0.18	-0.07	-0.47	-0.69	-0.40	0.64	0.50	0.69
	ARM 3	-0.11	-0.17	-0.17	-0.77	-0.68	-1.01	0.44	0.51	0.32
	SAQ 2	0.59	-0.10	0.64	5.22	-0.38	4.93	0.00	0.71	0.00
	CTQ 1	0.13	0.33	0.08	0.97	1.34	0.47	0.34	0.20	0.64
	CTQ 2	-0.11	0.16	-0.05	-0.80	0.62	-0.31	0.43	0.54	0.76
	CTQ 3	0.08	0.25	0.05	0.59	1.01	0.32	0.56	0.33	0.75
	CTQ 4	-0.14	0.33	-0.18	-1.03	1.33	-1.08	0.31	0.20	0.29
	CTQ 5	-0.10	0.36	-0.15	-0.73	1.48	-0.90	0.47	0.16	0.37
SAQ 2	*SPF 3*	-0.31	-0.29	-0.24	-2.36	-1.19	-1.45	0.02	0.25	0.15
	ARM 2	-0.06	-0.05	-0.09	-0.40	-0.20	-0.53	0.69	0.85	0.60
	ARM 3	-0.12	-0.19	-0.09	-0.85	-0.76	-0.51	0.40	0.46	0.61
	CTQ 1	0.13	0.14	0.09	0.98	0.53	0.56	0.33	0.60	0.58
	CTQ 2	-0.05	0.25	0.01	-0.33	1.00	0.07	0.74	0.34	0.95
	CTQ 3	-0.01	-0.10	0.06	-0.06	-0.38	0.34	0.95	0.71	0.73
	CTQ 4	-0.08	0.08	0.00	-0.57	0.30	-0.02	0.57	0.77	0.98
	CTQ 5	-0.19	-0.14	-0.11	-1.37	-0.54	-0.63	0.18	0.60	0.54

SAQ 3	*SPF 3*	-0.18	-0.32	-0.04	-1.30	-1.31	-0.22	0.20	0.21	0.82
	ARM 2	0.01	-0.16	0.08	0.07	-0.64	0.45	0.95	0.53	0.65
	ARM 3	-0.14	-0.48	0.00	-1.05	-2.13	0.01	0.30	0.05	0.99
	SAQ 2	0.31	0.31	0.29	2.37	1.25	1.77	0.02	0.23	0.09
	CTQ 1	0.00	0.19	-0.14	0.02	0.73	-0.84	0.98	0.47	0.41
	CTQ 2	0.05	0.30	0.04	0.38	1.24	0.21	0.70	0.24	0.84
	CTQ 3	0.07	0.22	0.00	0.53	0.88	-0.01	0.60	0.39	0.99
	CTQ 4	-0.16	0.07	-0.21	-1.15	0.27	-1.28	0.26	0.79	0.21
	CTQ 5	0.09	0.28	0.08	0.68	1.14	0.45	0.50	0.27	0.66
SAQ 4	*SPF 3*	-0.18	-0.32	-0.04	-1.30	-1.31	-0.22	0.20	0.21	0.82
	ARM 2	0.01	-0.16	0.08	0.07	-0.64	0.45	0.95	0.53	0.65
	ARM 3	-0.14	-0.48	0.00	-1.05	-2.13	0.01	0.30	0.05	0.99
	SAQ 2	0.31	0.31	0.29	2.37	1.25	1.77	0.02	0.23	0.09
	CTQ 1	0.00	0.19	-0.14	0.02	0.73	-0.84	0.98	0.47	0.41
	CTQ 2	0.05	0.30	0.04	0.38	1.24	0.21	0.70	0.24	0.84
	CTQ 3	0.07	0.22	0.00	0.53	0.88	-0.01	0.60	0.39	0.99
	CTQ 4	-0.16	0.07	-0.21	-1.15	0.27	-1.28	0.26	0.79	0.21
	CTQ 5	0.09	0.28	0.08	0.68	1.14	0.45	0.50	0.27	0.66
SAQ 5	*SPF 3*	-0.41	-0.36	-0.40	-3.28	-1.48	-2.57	0.00	0.16	0.01
	ARM 2	-0.02	0.00	-0.04	-0.16	0.00	-0.21	0.87	1.00	0.84
	ARM 3	-0.27	-0.22	-0.30	-2.05	-0.88	-1.89	0.04	0.40	0.07
	SAQ 2	0.57	0.77	0.50	4.97	4.68	3.43	0.00	0.00	0.00
	CTQ 1	0.04	0.01	0.01	0.25	0.04	0.07	0.80	0.97	0.94
	CTQ 2	0.01	0.38	-0.07	0.09	1.61	-0.43	0.93	0.13	0.67
	CTQ 3	0.09	0.21	0.03	0.62	0.82	0.16	0.54	0.42	0.87
	CTQ 4	-0.09	0.20	-0.15	-0.67	0.80	-0.88	0.51	0.44	0.38
	CTQ 5	0.05	0.09	0.08	0.37	0.34	0.45	0.71	0.74	0.66

CTQ 1	*SPF 3*	-0.04	0.09	-0.04	-0.32	0.36	-0.22	0.75	0.72	0.83
	ARM 2	-0.42	-0.31	-0.48	-3.38	-1.24	-3.23	0.00	0.23	0.00
	ARM 3	-0.27	-0.24	-0.26	-2.01	-0.96	-1.57	0.05	0.35	0.13
	SAQ 2	0.13	0.14	0.09	0.98	0.53	0.56	0.33	0.60	0.58
	CTQ 2	0.19	0.51	0.08	1.42	2.31	0.45	0.16	0.04	0.65
	CTQ 3	0.53	0.33	0.60	4.52	1.33	4.43	0.00	0.20	0.00
	CTQ 4	0.45	0.43	0.47	3.65	1.87	3.16	0.00	0.08	0.00
	CTQ 5	0.23	0.27	0.23	1.68	1.10	1.42	0.10	0.29	0.16
CTQ 2	*ARM 2*	0.01	-0.01	0.03	0.07	-0.04	0.16	0.95	0.97	0.88
	ARM 3	-0.12	-0.26	-0.09	-0.89	-1.04	-0.53	0.38	0.32	0.60
	SAQ 2	-0.05	0.25	0.01	-0.33	1.00	0.07	0.74	0.34	0.95
	CTQ 3	0.21	0.37	0.18	1.53	1.53	1.09	0.13	0.15	0.28
	CTQ 4	0.10	0.35	-0.08	0.71	1.46	-0.48	0.48	0.16	0.63
	CTQ 5	0.12	0.26	0.06	0.84	1.05	0.36	0.41	0.31	0.72
CTQ 3	*SPF 3*	-0.02	-0.29	0.12	-0.11	-1.19	0.74	0.91	0.25	0.46
	ARM 2	-0.33	-0.60	-0.18	-2.50	-2.90	-1.10	0.02	0.01	0.28
	ARM 3	-0.31	-0.43	-0.23	-2.33	-1.87	-1.37	0.02	0.08	0.18
	SAQ 2	-0.01	-0.10	0.06	-0.06	-0.38	0.34	0.95	0.71	0.73
	CTQ 4	0.22	0.57	0.07	1.62	2.71	0.42	0.11	0.02	0.68
	CTQ 5	0.08	0.50	-0.06	0.61	2.22	-0.37	0.55	0.04	0.71
CTQ 4	*SPF 3*	-0.19	-0.51	-0.13	-1.43	-2.32	-0.81	0.16	0.04	0.43
	ARM 2	-0.65	-0.69	-0.66	-6.21	-3.65	-5.24	0.00	0.00	0.00
	ARM 3	-0.42	-0.50	-0.39	-3.32	-2.25	-2.53	0.00	0.04	0.02
	SAQ 2	-0.08	0.08	0.00	-0.57	0.30	-0.02	0.57	0.77	0.98
	CTQ 5	0.46	0.40	0.50	3.69	1.67	3.44	0.00	0.12	0.00

CTQ 5	*SPF 3*	-0.16	-0.33	-0.14	-1.18	-1.37	-0.82	0.24	0.19	0.42
	ARM 2	-0.34	-0.24	-0.40	-2.60	-0.97	-2.61	0.01	0.35	0.01
	ARM 3	-0.20	-0.26	-0.27	-1.49	-1.05	-1.67	0.14	0.31	0.10
	SAQ 2	-0.19	-0.14	-0.11	-1.37	-0.54	-0.63	0.18	0.60	0.54

Appendix VI

Table 3.1 Raw scoring for resilience scales with respective genotypes

		SNP						SCALE OF PROTECTIVE FACTORS					BRS	ADULT RESILEICE MEASURE			
Sample ID	Sex	rs4633	rs680	rs4818	rs6269	rs737865	rs2075507	SPF 1	SPF 2	SPF 3	SPF 4	TOTAL SPF	TOTAL BRS	ARM 1	ARM 2	ARM 3	TOTAL ARM
83	M	C	A	C	G	A	-	3.33	5.33	4.5	3.83	17	2.83	43	25	35	103
85	M	C	A	C	G	A	A	4.5	3.5	5.5	2.33	15.83	3.83	47	19	32	98
90	F	C	G	C	G	A	A	3.83	4.33	6.67	7	21.83	3.33	55	34	50	139
92	M	C	A	C	G	A	A	5	5.5	6.83	6.17	23.5	2.5	47	31	44	122

Table 3.2 Raw scoring for anxiety scales with respective genotypes

	SNP						SOCIAL ANXIETY QUESTIONAIRRE						ADULT RESILEICE MEASURE		BECK ANXIETY INVENTORY
SAMPLE ID	rs4633	rs680	rs4818	rs6269	rs7378 65	rs2075 507	SAQ 1	SAQ 2	SAQ 3	SAQ 4	SAQ 5	TOTAL SAQ	STATE	TRAIT	TOTAL BAI
83	C	A	C	G	A	-	22	19	18	18	21	98	19	26	35
85	C	A	C	G	A	A	20	24	28	28	26	126	21	25	54
90	C	G	C	G	A	A	20	22	28	28	23	121	26	25	39
92	C	A	C	G	A	A	20	20	19	19	19	97	20	15	35

Table 3.3 Raw scoring for childhood trauma scales with respective genotypes

	SNP						CHILDHOOD TRAUMA QUESTIONNAIRE						ACE
SAMPLE ID	rs4633	rs680	rs4818	rs6269	rs737865	rs2075507	CTQ 1	CTQ 2	CTQ 3	CTQ 4	CTQ 5	TOTAL CTQ	TOTAL ACE
83	C	A	C	G	A	-	7	7	5	12	8	39	0
85	C	A	C	G	A	A	16	9	19	11	9	64	7
90	C	G	C	G	A	A	7	7	5	5	9	32	1
92	C	A	C	G	A	A	7	5	5	6	6	29	2